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The Effect Of Hypoxia And Simulated Exercise On Plasma Enzyme Activity In Dogs

Daniel John Loegering

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THE EFFECT OF HYPOXIA AND SIMULATED EXERCISE ON PLASMA ENZYME ACTIVITY IN DOGS

by

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ABSTRACT

This study was designed to determine if the relative hypoxia that exists in exercising muscles is responsible for the plasma enzyme response to exercise. The level of hypoxia that would cause a release of enzymes into the blood was determined by ventilating paralyzed anesthetized animals with gas mixtures containing known amounts of oxygen. Adult mongrel dogs were ventilated with 12, 10, 8.5, 7.5 percent oxygen (balance nitrogen) for 30 minutes or five percent oxygen for 15 minutes. Blood samples were taken from the right atrium and carotid artery for blood oxygen and carbon dioxide determinations. Plasma enzyme levels, blood pressure, heart rate and ECG (lead II) were obtained before, at the end of, and for six hours after the hypoxia. The enzymes studied were: glutamic-oxalacetic transaminase (GOT), creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and LDH isoenzymes.

The threshold for GOT release was between the level of hypoxia produced by 7.5 and 8.5 percent oxygen (11 mmHg and 17 mmHg arterial oxygen tension). The enzymes, CPK and LDH, had thresholds for release between the level of hypoxia produced by 10 and 12 percent oxygen (24 mmHg and 31 mmHg arterial oxygen tension). Thus, CPK and LDH

are released into the blood at similar levels of hypoxia and both are released with less severe levels of hypoxia than GOT.

A second series of experiments were performed to determine the effect of simulated exercise on plasma enzyme activity. These experiments were carried out so that plasma enzyme and blood gas changes during hypoxia and exercise could be compared under similar experimental conditions. Exercise was simulated by electrically stimulating the muscles of the hind legs of anesthetized dogs at five or 10 pulses per second for 30 minutes. Gas tensions were determined in femoral venous blood to estimate the degree of hypoxia associated with muscle stimulation. Stimulation at a rate of five pulses per second resulted in no change in plasma enzyme activity. Stimulation at a rate of 10 pulses per second caused an increase in PGOT and PLDH activity. Although PCPK and PLDH are equally sensitive to hypoxia there was no increase in PCPK activity with simulated exercise. PGOT and PLDH showed a much more transient response to simulated exercise than to hypoxia.

Previous studies have shown that prednisolone (a synthetic glucocorticoid) or nitroglycerin (a vasodilating agent) are capable of reducing the PGOT and PCPK response to exercise. Animals pre-treated with prednisolone (4 mg/kg) or given nitroglycerin during the hypoxia (0.06 mg/kg, intravenously at 10 minute intervals) showed no reduction in the PGOT or PCPK response to 7.5 percent oxygen. Animals given

nitroglycerin showed a reduced PLDH response to hypoxia. This reduction was due to a decrease in the amount of this enzyme released from the heart as indicated by the PLDH isoenzyme ratio.

It was concluded from this study that hypoxia was not the major cause of the plasma enzyme response to exercise.

I. INTRODUCTION

Exercise imposes a great physiological stress on both the circulatory system and on the muscles involved. One aspect of an organism's response to the stress of exercise is the increased level of activity of certain tissue enzymes in the blood. The degree of elevation following exercise is modified by the intensity and duration of exercise, as well as by the general level of physical fitness of the subject.

The cause of the increased rate of release of enzymes into the blood during and following exercise has not been elucidated despite extensive study of this phenomenon. The release of enzymes is caused by some change in cellular integrity (cellular necrosis or membrane permeability changes), however, the cause of this change has not been isolated.

Exercise does not cause a change in the arterial oxygen tension but there is a reduction in the venous oxygen tension. The increased need for oxygen in exercising muscles is met first by an increased extraction of oxygen from the blood and secondarily by an increase in blood flow. The decrease in venous oxygen tension indicates that the

muscle cells have a reduced oxygen tension and when this becomes extensive, the muscle cells are in what may be called a state of relative hypoxia. It is this relative hypoxia that may cause some loss of muscle cell integrity which would result in the efflux of enzymes into the blood.

Hypoxia is known to cause a release of enzymes, however, studies to date have been largely qualitative in nature and have involved rather strenuous experimental conditions or uncontrolled levels of hypoxia. The primary purpose of this study was to determine if the relative hypoxia that exists in exercising muscles is responsible for the plasma enzyme response to exercise. This was accomplished by ventilating paralyzed, anesthetized animals with gases containing known concentrations of oxygen and following the plasma enzyme response to hypoxia for several hours thereafter. The degree of hypoxia was assessed by measuring arterial and venous oxygen tensions. In a separate series of experiments, the leg muscles of anesthetized dogs were electrically stimulated to simulate the relative hypoxia that exists in exercising muscles. The intensity of the exercise caused by the electrical stimulation was assessed by measuring oxygen consumption. Changes in plasma enzymes and oxygen tension in the blood during these experiments allowed an estimation of the contribution that hypoxia makes to the plasma enzyme response to exercise.

A secondary purpose of this study was to determine the tissue or tissues that are most sensitive to hypoxia in terms of enzyme release.

If the plasma enzyme response to exercise is due to hypoxia in the exercising muscles, it seems logical that these muscles should be the major source of the enzymes released during exercise.

The source of the enzymes released after hypoxia was determined by measuring plasma glutamic-oxalacetic transaminase (PGOT), plasma creatine phosphokinase (PCPK), plasma lactate dehydrogenase (PLDH) and PLDH isoenzymes simultaneously in arterial, femoral venous, hepatic venous and right atrial blood. These sampling sites, and the tissue specificity of PCPK and PLDH isoenzymes, allowed the determination of the tissue or tissues that were the most sensitive to hypoxia in terms of enzyme release.

To further elucidate the mechanism of the plasma enzyme response to exercise, this study determined if drugs that decrease the plasma enzyme response to exercise would also decrease the plasma enzyme response to hypoxia. In 1968, Wagner and Critz found that three milligrams per kilogram of prednisolone (a synthetic glucocorticoid) administered orally to dogs before a one hour run on a treadmill decreased the PCPK increase following the exercise from five-fold to three-fold. Nerdrum and Nordøy (1964) found that nitroglycerin (a vasodilating agent) given to coronary patients immediately following exercise abolished the PGOT response to exercise seen in similar patients not given the drug. The administration of nitroglycerin to dogs during treadmill exercise reduced the PCPK increase following the

exercise (Wagner, 1967). If prednisolone and nitroglycerin decrease the plasma enzyme response to hypoxia, then the plasma enzyme response to exercise and hypoxia may be caused by similar mechanisms.

II. HISTORICAL REVIEW

(A) The Enzymes Studied: Glutamic-Oxalacetic Transaminase (GOT); Creatine Phosphokinase (CPK); Lactate Dehydrogenase (LDH); and LDH Isoenzymes

A large number of tissue enzymes are found in the blood in very small amounts and have been studied in both pathological and exercise situations. Three of these enzymes are included in this study: glutamic-oxalacetic transaminase (GOT), creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and LDH isoenzymes. These enzymes are appropriate for a study dealing with hypoxia and exercise since they have been investigated previously under conditions that will allow a comparison of results, and because of their important role in the metabolism of the heart and skeletal muscle.

GOT catalyzes the reversible exchange of the alpha-amino group of aspartic acid for the alpha-keto group of alpha-ketoglutaric acid resulting in the formation of glutamic acid and oxalacetic acid:

aspartic acid + α-ketoglutaric acid $\xleftrightarrow{\text{GOT}}$ glutamic acid + oxalacetic acid.

GOT is capable of interconverting these amino acids and keto acids with the formation of needed substrates as the metabolic situation demands.

(Cohen, 1942). Such an enzyme may be vital in providing a supply of metabolic substrates under conditions such as exercise. Most tissues contain this enzyme, but it is found in the highest concentration in skeletal muscle, brain, liver and heart (Nagode, Frajola and Loeb, 1966).

CPK catalyzes the reversible transfer of a high energy phosphate group from ATP to creatine as follows:

$$\text{ATP} + \text{Creatine} \xleftrightarrow{\text{CPK}} \text{ADP} + \text{Creatine phosphate}$$

This enzyme enables a tissue to store high energy phosphate as creatine phosphate which can be rapidly transferred to ADP as the metabolic demands dictate. Such an enzyme is very important in tissues such as skeletal muscle which require large amounts of ATP during periods of physical exertion. CPK is found in highest concentration in skeletal muscle with substantial amounts present in heart and brain tissue. There is no detectable CPK activity in kidney, liver or red blood cells (Hess, MacDonald, Frederick, Jones, Nely, and Gross, 1964).

LDH catalyzes the reversible conversion of pyruvic acid to lactic acid with the oxidation of NADH to NAD:

$$\text{Pyruvic acid} + \text{NADH} \xleftrightarrow{\text{LDH}} \text{lactic acid} + \text{NAD}$$

This enzyme is important for anaerobic metabolism in that it allows the limited amounts of NAD to be recycled for glycolysis when there is a

lack of oxygen. LDH is found in highest concentration in skeletal muscle, liver, heart and brain with lesser amounts in most of the other tissues of the body (Nagode et al. 1966).

Isoenzymes are proteins with the same enzymatic specificity that can be separated into different molecular forms by physiochemical methods. There are two isoenzymes of GOT which can be separated according to their intracellular location (mitochondria or cytoplasm). These two isoenzymes are identical in the various tissues that contain GOT activity (Boyd, 1961). There are also two isoenzymes of CPK. Heart muscle contains both isoenzymes while skeletal muscle contains only one and brain tissue contains only the other isoenzyme (Sjovall and Voight, 1964; Deul and Van Breeman, 1964). LDH isoenzyme distribution is more complex.

The LDH isoenzyme is made up of four subunits of which there are two types. These two different subunits are found in all possible combinations of four, giving five different LDH isoenzymes. The LDH isoenzyme found in heart muscle is made up predominantly of what is known as H subunits giving a configuration of H4; and isoenzymes found in the skeletal muscle and liver are made up predominantly of M subunits (M4). LDH isoenzymes found in other tissues are made up of other combinations of the H and M subunits (H_3M_1, H_2M_2, H_1M_3) Wilson, Chan and Kaplan, 1963). The tissue specificity of the LDH isoenzymes can be used to determine the source of the enzyme in the serum. All

five of the isoenzymes are normally found in the serum and if one of the isoenzymes is abnormally elevated the tissue or tissues in which it is found is releasing LDH into the blood at an increased rate.

(B) The Plasma Enzyme Response to Exercise

The plasma enzyme response to exercise has been studied extensively. The purpose of this review is to give a description of the response of PGOT, PCPK, PLDH, and PLDH isoenzymes and is not a comprehensive review of all the work done in this area. The early work on the plasma enzyme response to exercise was largely of a qualitative nature (Henley, Schmidt and Schmidt, 1960; Schlang and Kirkpatrick, 1961; Tessari and Parrini, 1961) in that these studies merely stated that exercise could cause an increase in plasma enzyme levels. At the same time, a discrepancy arose as to the extent of cellular disruption needed to cause a detectable release of enzymes during exercise. Henley et al (1960) stated that an elevation of PGOT activity caused by exercise was not associated with cell necrosis. However, the following year, Altland and Highman (1961) demonstrated clearly that rats exercised for 16 hours showed PGOT and PLDH increases which were associated with histological changes in muscle tissue. These authors concluded that plasma enzyme changes with exercise are associated with tissue necrosis. This problem has not been resolved to date.

More recent studies have indicated that both the duration and intensity of the exercise dictate the magnitude of the enzyme response.

Fowler, Chowdhury, Pearson, Gardner and Bratton (1962) found that subjects running on a treadmill (five miles per hour, 15 minutes, at 3°, 5°, 8° or 11° grade) showed a progressive increase in PGOT and PLDH activity as the grade was increased. In 1963, Highman and Altland found a linear increase in PGOT and PLDH activity in rats walking in a rotating drum for 6, 12 and 16 hours. On the other hand, Critz (1966) demonstrated that PGOT activity in swimming rats increased progressively after one-half hour and one hour of exercise, but with little further increase after two hours of exercise. Subjects walking 25 miles had a seven-fold increase in PCPK activity (Griffiths, 1966) but running up and down two flights of stairs three times caused no change in PCPK activity (Pearce, Pennington and Walton, 1964).

An interesting aspect of the plasma enzyme response to exercise is that the individual enzymes show differing responses to different types of exercise. According to Halonen and Konttinen (1962) marching for two hours had no effect on PGOT activity but PLDH activity was significantly increased. Nerdrum and Berg (1964) found that short term exercise (sprinting 100 to 200 meters or a 100 meter swim) caused an increase in PGOT activity with no change in PLDH activity; while long term exercise (15 kilometers march or performing calisthenics for 90 minutes) caused no change in PGOT activity and an increase in PLDH activity. A 25 mile walk caused a seven-fold increase in PCPK activity (Griffiths, 1966). A maximal oxygen consumption test on a bicycle

ergometer caused a greater increase in PLDH activity than in PGOT or PCPK activity (Fowler, Gardner, Kazerunian and Lauvstad, 1968). The same study showed that a 440 yard dash increased PGOT activity 140 percent but there was no change in PLDH activity. An eight mile run increased PGOT activity 75 percent and PLDH activity increased 35 percent, and following a maximal treadmill exercise of 15 minutes, PGOT activity increased 60 percent and PLDH activity increased 30 percent. Dogs running on a treadmill for one hour at 10 miles per hour had a five-fold increase in PCPK (Wagner and Critz, 1968) while brief maximal exercise on a bicycle ergometer caused only a slight increase in PCPK activity (Fowler et al, 1968). In summary, it appears that brief, maximal exercise has more of an effect on PGOT than on PLDH or PCPK; mild exercise of long duration has no effect on PGOT but PLDH and PCPK increase. Heavy exercise, if of sufficient duration, causes all three enzymes to increase.

The effect of exercise on LDH isoenzymes in plasma was first investigated by Garbus, Highman and Altland, in 1964. This study showed that rats walking in a rotating drum for 16 hours had an increase in the activity of all five PLDH isoenzymes. This indicates that during exercise the increased PLDH activity is the result of a loss of LDH from skeletal muscle as well as substantial contributions from the heart and the other organs. Papadopoulos, Leon and Bloor (1967; 1968) confirmed these results in rats swimming for four hours.

The length of time necessary following exercise for the plasma enzymes to return to pre-exercise levels appears to depend upon the magnitude of the increase. Altland and Highman (1961) found that rats exercised for 16 hours, which increased the PGOT activity about five-fold, required 72 hours to return to normal. The PLDH activity, which increased only two-fold, returned to control levels within 24 hours. Two hours of marching caused PLDH activity to increase about 60 percent and returned to control levels within two hours (Holonen and Konttinen, 1962). Wagner and Critz (1968) found a five-fold increase in PCPK activity in dogs immediately following a one hour run on a treadmill. Thirty minutes after exercise the PCPK activity showed a slight further increase. In 1964, Nerdrum and Berg found that brief, maximal effort was associated with a 50 percent increase in PGOT activity which returned to normal within 45 minutes. However, the PLDH activity increase (30 percent) seen after prolonged exercise had increased still further 45 minutes after the exercise. A 15 minute run on a treadmill (five miles per hour, 11° grade) caused a 50 percent increase in PGOT activity and a 20 percent increase in PLDH activity. Both enzymes returned to control levels within 15 minutes after the exercise (Fowler, et al, 1962). In 1968, Fowler et al showed that a brief, maximal effort on a bicycle ergometer caused an increase in PCPK activity which returned to near normal in five minutes.

(C) The Effect of Simulated Exercise on Plasma Enzyme Activity

Few studies have been carried out to determine the effect of electrical stimulation of skeletal muscles on plasma enzyme activities. Lending, Slobody and Mestern (1959) found that metrazol or electro-shock convulsions lasting for 30 minutes caused the PLDH and PGOT activity to more than double in puppies. In 1968, Hunt and Bailie found that direct current shock for atrial fibrillation caused powerful inter-costal muscle contractions and an increase in PCPK activity but with no change in PGOT activity. A marked and prolonged increase in PCPK activity was seen in rabbits subjected to 50 volts at 50 c/sec. for five seconds (Guglielmetti, Tominz and Andreuzzi, 1965). Likewise, direct stimulation of skeletal muscle resulted in an increase in PCPK activity (Maksimova, 1966).

(D) The Effect of Physical Training on the Plasma Enzyme Response to Exercise

The fact that training can modify the plasma enzyme response to exercise was first demonstrated by Fowler et al (1962). Treadmill exercise (15 minutes) caused a greater increase in PGOT and PLDH in the untrained than in highly trained subjects. In 1963, Highman and Altland found that rats walking in a rotating drum for six hours per day showed a progressive increase in the PGOT activity after the exercise for the first three days. As the training continued, there was a progressive decrease in the PGOT response to the exercise until after six

days of training there was no increase in PGOT activity. This same degree of training did not alter the PLDH response. The following year, Garbus et al (1964) found that three weeks of training, six hours per day, was required to abolish the PLDH response to 16 hours of exercise. This training regimen also abolished the PGOT response. Papadopoulos et al (1968) using swimming exercise to train rats found that swimming for one hour per day for one week had little effect, while training for one hour per day for ten weeks reduced the PLDH response to a four hour swim by about one-half. When the daily swim was lengthened to four hours the training regimen could be reduced to five weeks and obtain the same effect on the PLDH response as was seen with one hour per day swimming for ten weeks. Ten weeks of training for four hours per day was required to completely abolish the PLDH response to four hours of swimming. Ahlborg and Brohult (1967) found that 90 minutes of strenuous exercise on a bicycle ergometer caused a greater increase in PGOT, PLDH and PCPK activity in untrained subjects, and required longer to return to normal than in highly trained subjects. In 1968, Nuttall and Jones found that six minutes of lifting weights equal to 15 percent of the subjects body weight caused an increase in PGOT and PCPK activity. This increase was abolished if the subjects lifted weights for six minutes a week for three to five weeks.

Bloor and Papadopoulos (1969) studied the response of rats to exercise for different periods following cessation of training. These

authors found that four to seven weeks after training (swimming four hours per day for ten weeks) there was an increase in PLDH activity after exercise that was about one-half as great as in untrained rats. It was concluded that the complete absence of PLDH changes following exercise in rats trained for ten weeks is not permanent and as the animals become deconditioned the PLDH response to exercise returned to that seen in untrained animals.

The effect of training on the LDH isoenzyme response to exercise was studied by Papadopoulos et al (1968). As shown previously, all of the isoenzymes were increased following exercise in untrained animals. After two to five weeks of swimming four hours per day, rats responded to a four hour swim with greater increases in fractions one and two (heart and kidney) than in fraction five (liver and skeletal muscle). After ten weeks of such training the response to exercise was an isoenzyme pattern similar to unexercised controls. The following year Bloor and Papadopoulos (1969) found that seven weeks following a ten week training program the isoenzyme response to exercise showed an increase in only fraction five. These results indicate that during training heart and kidney take longer to become trained and stay trained longer than liver and skeletal muscle with regard to the LDH release.

(E) The Effect of Exercise and Training on Tissue Enzyme Activity

The effect of exercise and training on tissue enzyme activity has

not been studied as extensively as the effect of exercise and training on plasma enzyme activity. Gollnick, Struck and Bogyo (1967) found that 30 minutes of swimming did not change the LDH activity in skeletal muscle or heart muscle of rats. Papadopoulos et al (1967) found similar results in rats swimming for four hours. However, men engaged in a two hour competitive cross country run, or four hours on a treadmill at 70 percent maximum load had a 21 percent increase in the LDH activity of the quadriceps muscle (Karlsson, Diamant and Saltin, 1968). Kindrick-Jones and Perry (1965) found that 20 hours of exercise on a treadmill caused an increase in the CPK activity in skeletal muscle of rats. In 1964, Critz and Merrick found an increase in heart and skeletal muscle GOT activity in rats after one hour of swimming exercise. The following year, Critz and Withrow (1965) demonstrated that adrenalectomized rats had no change in heart or skeletal muscle GOT activity following a one hour swim. These authors concluded that adrenal corticoids are essential for the rise in GOT activity in heart and skeletal muscle associated with exercise.

The effect of training on tissue enzyme activity was studied by Gould and Rawlinson (1959). Six weeks of swimming for 30 minutes a day, five days a week did not change the skeletal muscle LDH activity. Using a similar training regimen, Gollnick and Hearn (1961) found an increase in heart LDH activity and again, no change in skeletal muscle LDH activity. In 1967, Gollnick, Struck and Bogyo intensified the

training to one hour swimming per day for 35 days. This resulted in a decrease in skeletal muscle, and an increase in heart LDH activity in rats. Chronic elevation of arterial blood pressure induced by injections of desoxycorticosterone acetate (Critz, 1963) or coarctation of the abdominal aorta (Critz and Withrow, 1964) caused a rise in GOT activity in the left ventricles of rats. On the other hand, Kindrick-Jones and Perry (1965) found that three weeks of restricted activity reduced the CPK activity of skeletal muscle in rats.

In 1966, Garcia-Buñuel, Garcia-Buñuel, Green and Subin found that immobilization of a hind limb of rabbits resulted in reduction of LDH activity in the soleus muscle. These authors also found that denervation of a hind limb caused a reduction in LDH activity of the soleus and gastrocnemius muscles of rabbits. Similarly, there is a reduction in the GOT activity of the tibialis anterior muscle of the rabbit after denervation (Flodmark, Jungner and Petersén, 1959).

(F) The Effect of Hypoxia on Plasma Enzymes

The first study on the effect of hypoxia on serum enzymes was published in 1957 by Merrill, Lemley-Stone and Meneely. These authors found that rats exposed to seven percent oxygen for one hour, had no increase in PGOT activity. Later, Asvall (1960) showed that rabbits exposed to 3.8 percent oxygen for 30 minutes had PGOT activity more than double the control level immediately following the hypoxia. There was no further increase three hours later and the PGOT activity

returned to the control level within 20 hours following the hypoxic episode. The same year Highman and Altland (1960) found that dogs exposed to a simulated altitude of 32,000 feet (206 mmHg) for four hours had more than a three-fold increase in PGOT activity while PLDH activity more than doubled. Human subjects exposed to 22,700 feet (312 mmHg) for 30 minutes had a 10 percent increase in PGOT activity which returned to control levels within one hour (Wegmann, Bruner, Klein and Voigt, 1966).

In 1961, Highman and Altland found that dogs exposed to 32,000 feet (206 mmHg) for four hours, five days a week for seven weeks had a progressive decrease in PGOT and PLDH response following the exposure, but there was a progressive increase in the pre-exposure levels. The increase in pre-exposure levels indicates that there was not sufficient time between exposures for the enzymes to return to normal. After the final exposure six weeks were required for the PGOT and PLDH activities to return to control levels.

Altland, Highman and Garbus (1964) found that rats exposed to 28,000 feet (247 mmHg) for six hours had an increase in PGOT and PLDH activity. Rats exercising at this altitude showed greater increases in plasma enzymes. However, rats trained at sea level six hours per day for three days and then exercised at 28,000 feet had less increase in PGOT and PLDH activity than rats exercising at altitude without training. Training for one to three weeks had little more effect

in reducing the response than training for three days when the animals were made to exercise at 28,000 feet. Although the training prevented the additional increase seen in animals exercised at 28,000 feet, the training did not decrease the response below that initially seen in untrained animals exposed to 28,000 feet.

In 1966, Highman and Altland found that rats exposed to various simulated altitudes for five hours had an increase in PGOT and PLDH after exposure to 25,000 feet (282 mmHg). However, exposure to 22,000 feet (321 mmHg) caused an increase in PLDH activity only. Refsum (1963) studying patients with pulmonary insufficiency observed an increase in PGOT and PLDH activity when the arterial oxygen content fell below nine volumes percent, and an increase in only PLDH when the arterial oxygen content fell below 12 volumes percent. Another study showed that some patients with various types of pulmonary diseases had increased PCPK activity but all patients had normal PGOT activity (Vélez-García, Hardy, Dioso and Perkoff, 1966). These results indicate that changes in PLDH and PCPK may be more sensitive to hypoxia than PGOT.

Hypoxia and exercise cause a similar change in the LDH isoenzymes. Altland et al (1964) and Highman and Altland (1966) found little change in the proportions of the LDH isoenzymes in the plasma of rats after exposure to 28,000 feet (247 mmHg) for five or six hours. Similar results were obtained by Selméci, Farkas, Pósch, Szelényi and

Séc (1967) in rats exposed to an altitude of 18,500 feet (374 mmHg) for six hours. These results indicate that many tissues release LDH into the blood during hypoxia.

A study by Nelson (1966) indicates that the plasma enzyme increases noted on exposure to hypoxia may be due to the release of lysosomal enzymes. In this study, it was shown that exposure to hypoxia in the form of simulated high altitude reduces the stability of rat hepatic lysosomes, and that with exposure to 32,000 feet (206 mmHg) the PGOT activity increase paralleled the increase in acid phosphatase released from lysosomes. At less extreme altitudes, 24,000 feet (294 mmHg) or 28,000 feet (247 mmHg), the rise in acid phosphatase preceded a comparable increase in PGOT activity. It was suggested the lysosomal enzymes may play an important role in initiating tissue damage with subsequent release of tissue enzymes into the blood.

The effect of hemorrhagic shock on PLDH activity was studied by Vesell, Feldman and Frank (1959). In this study, dogs were hemorrhaged until the arterial blood pressure reached 30 or 40 mmHg. The PLDH activity increased to 10 times the control values after four hours at 40 mmHg; and the PLDH activity increased from 15 to 50 times the control values after four hours at 30 mmHg. The magnitude of the increases in PLDH activity was related to the degree of deterioration of the preparation (as determined by the amount of blood taken up from a reservoir in order to maintain the arterial pressure). During the

experiments blood samples were taken from the femoral artery, femoral vein, hepatic vein and renal vein; all three of the venous samples consistently showed higher PLDH activity than the arterial sample. Again, these authors concluded that the LDH activity was derived from most of the tissues of the body. Shubin and Weil (1963) studied patients in circulatory shock (caused by acute hypotension) not complicated by myocardial infarction or liver disease and found that of 22 patients, 70 percent had increased PGOT activity and 52 percent had increased PLDH activity. The magnitude of the increase in plasma enzyme activity was correlated with the duration of shock.

(G) The Effect of Hypoxia on Tissue Enzyme Activity

The effect of acute exposure to hypoxia on tissue enzyme activity has not been studied extensively. Rats exposed to seven percent oxygen for one hour had a 27 percent decrease in heart GOT activity and a comparable decrease in skeletal muscle, liver and kidney GOT (Merrill et al, 1957). However, these animals had no change in PGOT activity. Blyuger, Belen'kii and Shuster (1965) found that rats exposed to 4,000 meters (462 mmHg) for two hours had a slight decrease in heart and liver GOT activity. LDH activity in heart, kidney and liver decreased in rats exposed to total anoxia for six minutes (Kim and Han, 1969).

(H) The Effect of Prednisolone on Tissue Enzyme Release

Prednisolone, a synthetic, dehydrogenated analogue of hydro-

cortisone, has typical glucocorticoid actions. These include: gluconeogenesis, hyperglycemia, increased liver glycogen, increased plasma amino acids, increased urinary nitrogen, accumulation of sodium and water, and an increased rate of synthesis of certain tissue enzymes.

The metabolic effects of administered glucocorticoids require several hours to several days to develop. Within 12 hours after administration liver glycogen and blood glucose is elevated and there is an increase in urinary nitrogen (Long, Katzin and Fry, 1940; Mills, 1965). The decrease in body weight that develops as a result of administration of large amounts of glucocorticoids is not seen for several days (Fielder, Hoff, Thomas, Talksdorf, Perlman and Cronin, 1959; Fraldi, Mills and Chayes, 1964).

Administration of glucocorticoids causes an increase in liver GOT activity (Puchol and Carballido, 1959), however, a latent period of more than two days is required for such changes to occur (Rosen, Roberts, Budnick and Nichol, 1958). Similar results have been found for liver LDH activity (Weber, Banerjee and Bronstein, 1961; 1962). Similarly, long term (10 to 14 days) administration of glucocorticoids will cause a slight elevation of PGOT activity (Bavetta, Bekhor, Shah, O'Day and Nimni, 1962; Gregorczyk, Stanosek and Lewandowska-Tokarz, 1965) and short term administration (two days) has no effect on the PGOT or PLDH activity (Schreiber and Lesch, 1965). Since this study is concerned with only the effects seen within 12 to 18 hours after prednisolone

administration, the ensuing discussion will be limited to investigations concerning the effect of prednisolone on the release of enzymes from tissues.

The ability of glucocorticoids to reduce enzyme loss from tissues has been demonstrated in a wide variety of experiments. Prednisolone administration to patients with viral hepatitis has been shown to be effective in rapidly returning PGOT activity towards normal (deRitis, Coltorti and Giuti, 1959). These authors concluded that the effect of prednisolone in this situation was due to a general improvement in liver cell metabolism since the decrease in PGOT paralleled the decrease in serum bilirubin. In 1961 Benda, Locker and Moser found that dogs pretreated with cortisone and given carbon tetrachloride showed a smaller and more transient increase in PLDH activity than animals not given cortisone. Histologic examination of the liver showed that the glucocorticoid did not decrease the extent of the cellular necrosis caused by carbon tetrachloride.

Lathritic rats were shown to have elevated PGOT activity (Korting, Holtzmann and Morsches, 1966). This was considered to be due to a general increase in cell membrane permeability and collagen breakdown. The increase in PGOT activity was abolished if prednisolone was included in the diet that produced latherism. In a similar study, Holtzmann, Korting and Morsches (1965) found that the elevation in PCPK activity in lathritic rats was decreased by 50 percent with prednisolone treatment.

Nakata, Suematsu and Sakamoto (1963) found that rats injected with tumor cells (strain AH 130) had an increase in PGOT and a decrease in liver GOT activity. Administration of hydrocortisone five hours before the animals were sacrificed abolished both of these changes.

It is well known that myocardial infarction will decrease the GOT activity in the infarcted area (Hess, 1963). This change was reduced, however, by administration of prednisolone either before or after ligation of a coronary artery in dogs (Huzino, Kimura, Aburaya and Katunuma, 1963).

Wagner and Critz (1968) found that oral administration of three milligrams per kilogram of prednisolone two hours prior to a one hour run on a treadmill reduced the PCPK increase following the exercise from five-fold to three-fold. These authors concluded that the protective effect of prednisolone (in terms of preventing enzyme loss from tissue) was due to the drug's ability to maintain the integrity of cell membranes.

Nelson (1966) has suggested that disruption of lysosomes with subsequent release of proteolytic enzymes may be one factor involved in enzyme release from tissues subjected to hypoxia. This suggestion was based on the fact that lysosomes isolated from altitude-exposed rats released acid phosphatase at an increased rate. Also, the increase in PGOT seen in rats exposed to various altitudes was preceded by an increase in nonsedimentable liver acid phosphatase.

The ability of prednisolone to stabilize lysosomes has been studied by Wissemann and Thomas (1962). They demonstrated that liver lysosomes taken from rats treated with hydrocortisone for four days released acid proteases at a reduced rate when exposed to mercury arc irradiation. Similarly, hydrocortisone added to suspensions of liver lysosomes caused a reduced release of acid hydrolases during exposure to mercury arc irradiation. Gordis and Nitowsky (1965) found that prednisolone added to the growth medium of cell cultures in physiologic amounts (one microgram per milliliter) was effective in reducing the acid phosphatase release from lysosome fractions during acid incubation.

The ability of prednisolone to decrease the release of enzymes from tissues under various conditions, including exercise; and its ability to stabilize lysosomes, makes this drug a logical choice for use in attempting to reduce the plasma enzyme response to hypoxia.

(I) The Effect of Nitroglycerin on Tissue Enzyme Release

Nerdrum and Nordøy (1964) studied the PGOT response to exercise in normal subjects and patients with coronary disease. The subjects exercised on a bicycle ergometer for three minutes at a work load of 300 kilogram-meters per minute. Normal subjects showed a slight decrease in PGOT activity following such exercise. One group of patients with coronary disease showed no change in PGOT activity and was able to complete the exercise without anginal pain or changes in

their ECG. Another group of patients, however, was unable to complete the exercise due to development of angina pectoris. All of these patients displayed ischemic ECG records and an increase in PGOT activity. The third group of patients was similar to the latter group in that they could not complete the exercise, but were given nitroglycerin immediately following the exercise. This group had no increase in PGOT activity.

Wagner (1967) studied the effect of nitroglycerin on the PCPK response to exercise in dogs. The animals were exercised on a treadmill for one hour at 10 miles per hour and nitroglycerin was administered intravenously every 15 minutes during the exercise. The control animals, which did not receive nitroglycerin, showed an increase in PCPK activity of 200 units while the animals receiving nitroglycerin showed an increase of only 100 units.

The mechanism of action of nitroglycerin in relieving anginal symptoms has been studied extensively. Until recently, it was believed that nitroglycerin increased the blood supply to the myocardium through vasodilitation of the coronary vessels. Several studies have shown that although an increase in total coronary blood flow may occur after the intravenous administration of nitroglycerine, the effect lasts for less than 90 seconds (Bergamaschi and Glasser, 1963; Bernstein, Barbier, Gold and Corday, 1963; Elliot and Heath, 1963; Melville, Gillis and Sekelj, 1965; Bernstein, Friesinger, Lichtlen and Ross, 1966). The

very transient nature of the coronary vasodilitation after nitroglycerin is due to the rapid reduction in arterial blood pressure. Melville, Gillis and Sekelj (1965) found that coronary blood flow increased to a maximum 10 seconds following the intravenous administration of nitroglycerin; at this time the arterial blood pressure was only slightly decreased. Twenty seconds following administration, the coronary blood flow had decreased to control levels at which time the arterial blood pressure was decreased maximally. Elliot and Heath (1963) were able to prolong the increase in coronary blood flow after intravenous nitroglycerin administration by maintaining a constant aortic blood pressure.

Nitroglycerin apparently reduces arterial blood pressure by its ability to decrease arteriolar and venous tone (Mason and Braunwald, 1965).

As early as 1963, Bernstein et al found that nitroglycerin temporarily reduced the tension-time index (the tension-time index correlates highly with myocardial oxygen consumption; Sarnoff, Braunwald, Welch, Case, Stainsby and Macruz, 1958). In 1965, Williams, Glick and Braunwald found that nitroglycerin also caused a decrease in the dimensions of both ventricles. A decrease in ventricular dimensions would reduce myocardial fiber tension and, in turn, decrease the oxygen requirement of the heart. Bernstein et al (1966) measured myocardial oxygen consumption and found that nitroglycerin did reduce the oxygen consumption of the heart. This action of nitroglycerin is

believed to be important for relieving anginal symptoms.

Robinson (1968) studied the effect of nitroglycerin on patients with angina pectoris during exercise. The patients exercised on a bicycle ergometer at a load that would cause anginal pain in less than five minutes. At the onset of pain the exercise was terminated and the patient was allowed to rest for 10 minutes. At the end of the rest period nitroglycerin (0.5 milligram) was given sublingually and the exercise was resumed after three minutes. The typical response to nitroglycerin during exercise was a reduction in systolic blood pressure, decreased ejection time and an increase in heart rate. There was a 17 percent reduction in the rate-pressure product (an index of the oxygen consumption of the heart). After nitroglycerin administration, the patients were able to exercise for a longer period of time, and at a higher work load. However, anginal symptoms still appeared when the rate-pressure product reached a critical value. In other words, anginal symptoms always appeared at the same rate-pressure product; nitroglycerin was capable of delaying the attainment of this critical value.

The ability of nitroglycerin to reduce the plasma enzyme response to exercise is probably due to a reduction in myocardial oxygen requirement during exercise. It seems logical, therefore, that this drug would decrease the plasma enzyme response to hypoxia.

III. METHODS AND MATERIALS

(A) Animals

Adult, mongrel dogs of either sex were used as experimental animals in this study. The mean weight was 15.8 kilograms with a range of 10 to 24 kilograms. The dogs were anesthetized with sodium pentobarbital (26.5 milligrams per kilogram, intravenously). Small additional doses were given through the course of the experiment as required.

(B) Experimental Design

Plasma enzyme activity of arterial blood samples was determined before, at the end of, and at one-half, one, two, four, and six hours after the hypoxic episode. Arterial and venous oxygen and carbon dioxide tensions, as well as blood pressure, heart rate and electrocardiogram (ECG) were also recorded at these times. Arterial blood samples were obtained from a "T" tube cannula in the left carotid artery. Venous blood samples were obtained from a catheter advanced down the external jugular vein to the right atrium. Arterial blood pressure was measured via the cannula in the carotid artery with a P23Dc Statham Pressure Transducer, and a standard lead II ECG was recorded with a Grass Model 7 Polygraph. Blood oxygen and carbon dioxide tensions were determined potentiometrically using a Model 113

Instrumentation Laboratory Gas Analyzer. Blood pressure, heart rate and ECG were recorded prior to, during the hypoxia and during the following six hours at the above times. The ECG was analyzed for ischemic changes according to the criteria of Asada, Chiba, Osawa, Nakamura and Murakawa (1962) (ST segment displacement of more than 0.1 mV or T wave flattening of more than 0.15 mV).

(C) Experimental Series

1. Hypoxia

Animals were ventilated with 12, 10, 8.5, 7.5 percent oxygen (balance nitrogen) for 30 minutes or with five percent oxygen for 15 minutes. The animals were ventilated with five percent oxygen for 15 minutes since preliminary studies indicated that dogs would not consistently survive 30 minutes of such treatment. The gas mixtures were prepared in a Tissot spirometer and were analyzed for oxygen tension. Mixtures that were within 0.2 percent of the desired level were used. The Tissot spirometer was connected to a positive pressure respirator and the animal was ventilated at an appropriate rate and depth via a trachea cannula for 30 minutes (15 minutes for five percent oxygen). Control animals were ventilated with room air for 30 minutes. Succinylcholine was administered intravenously during the hypoxia in amounts sufficient to paralyze the respiratory muscles and prevent attempted hyperventilation.

2. Simulated Exercise

Exercise was simulated by electrically stimulating the muscles of the hind legs. Four disc electrodes were attached to the skin of the thigh of both hind legs. The electrodes were attached half way down the thigh over the biceps femoris, semitendinosus, gracilis and sartorius muscles. A square wave pulse of 70 volts and 10 milliseconds duration was used at frequencies of five or 10 pulses per second for 30 minutes. Blood samples were taken from the right femoral vein by venipuncture and analyzed for oxygen and carbon dioxide tensions at the same times that blood gas analyses were carried out on the arterial and venous blood samples. Electrode placement for recording the ECG was similar to that used by Bellet, Marcel, Deliyiannis and Figallo (1962). One exploring electrode was placed on the fifth intercostal space, left mid-axillary line, and the second electrode just to the right of the anterior end of the sternum. The amount of work performed during the simulated exercise was estimated from oxygen consumption measurements carried out during, one-half hour and two hours after the stimulation. Oxygen consumption was determined by the closed circuit method using a Collins spirometer. It is well known that there is a linear relationship between oxygen consumption and the amount of work done during exercise (Morehouse and Miller, 1967).

3. Multiple Blood Sampling Sites

The tissues most sensitive to hypoxia in terms of enzyme

release were determined by analyzing plasma enzyme activity in samples taken simultaneously from four sites. These four sites were the: (a) left carotid artery; (b) right atrium; (c) right femoral vein; and (d) a catheter advanced up the inferior vena cava from the left femoral vein to the level of the hepatic veins. Samples taken from this catheter contained hepatic venous blood. At the conclusion of the experiments, the animals were sacrificed and the position of the catheter was verified. Plasma enzyme activity from all four sampling sites were determined only in dogs ventilated with five percent oxygen. Arterial and right atrial samples were analyzed for enzyme activity in dogs ventilated with 10 percent oxygen and in control dogs. In the remainder of the study only the arterial sample was analyzed for enzyme activity.

4. Prednisolone and Nitroglycerin

Prednisolone was administered orally (three milligrams per kilogram) 12 hours before the experiment and intravenously (one milligram per kilogram dissolved in 30 percent ethanol) one-half hour before the hypoxia. Nitroglycerin (glyceryl trinitrate) was administered intravenously (0.06 milligrams per kilogram) three times during the hypoxia (at 10 minute intervals). These drugs were administered to three groups of animals: (a) animals pre-treated with prednisolone and ventilated with 7.5 percent oxygen for 30 minutes; (b) animals given nitroglycerin while being ventilated with 7.5 percent oxygen; and (c) animals given both drugs and ventilated with room air (drug controls).

5. Succinylcholine

Succinylcholine was administered to all groups of animals during the hypoxia in amounts sufficient to paralyze the respiratory muscles. However, previous studies have shown that succinylcholine administration during anaesthesia may cause an increase in PCPK (Tammisto and Airaksinen, 1966; Tammisto, Leikkonen and Airaksinen, 1967). These authors suggested that the increase in PCPK may have been due to hypoxia which developed in muscles as a result of the depolarization of muscle cells caused by this drug. Although succinylcholine was administered to the control animals, a series of experiments were carried out in order to determine if this drug was modifying the plasma enzyme response to hypoxia. Succinylcholine was not administered to a series of animals ventilated with 10 percent oxygen for 30 minutes. This group was then compared with the series that had received succinylcholine while being ventilated with 10 percent oxygen.

(D) Enzyme Analysis

All blood samples were taken with heparinized syringes. Five milliliter samples drawn for enzyme analysis were centrifuged immediately for five minutes, the plasma separated and stored under refrigeration (5°C) until analyzed. GOT analyses were carried out within 18 hours and analysis for CPK and LDH activity was carried out within six hours. These enzymes are quite stable when refrigerated

for this length of time (Karmen, Wróblewski and LaDue, 1955; Nachlas, Margulies, Goldburg and Seligman, 1960; Nielsen and Ludvigsen, 1963).

GOT activity was determined according to the method of Karmen (1955) while CPK activity was determined according to the method of Oliver (1955). The reagents for these analyses were supplied by Calbiochem. The method of Babson and Phillips (1965) was used to determine LDH activity. PGOT, PCPK and PLDH activities were expressed in terms of international units (I.U.) per liter of plasma. LDH isoenzyme activity was separated into H and M subunit activity according to the method of Babson (1967). In this analysis, a one molar solution of urea is included in the reaction mixture to suppress M subunit activity and a one molar solution of lactate is used to suppress the H subunit activity. The H and M subunit activities are thus determined separately and expressed as the ratio of M/H (LDH isoenzyme ratio) which gives an indication of the proportion of plasma LDH activity that is due to the two isoenzyme subunits. The reagents for the PLDH and LDH isoenzyme analysis were supplied by Warner-Chilcott Laboratories.

(E) Statistical Analysis

Enzyme activity was expressed as the difference from the before hypoxia sample for purposes of statistical analysis. The mean and standard error of the mean of the raw enzyme data are given in

the Appendices. A two way repeated measures analysis of variance test was used to detect differences in the change in enzyme activity of the experimental groups as compared with the control groups (Winer, 1962). Enzyme data from the multiple sampling sites experiments were analyzed with a three way repeated measures analysis of variance test (Winer, 1962). The null hypothesis was rejected at the five percent level.

Blood gas, heart rate and blood pressure data were not subjected to statistical analysis. Mean and standard error of the mean of these data are given in the Tables.

IV. RESULTS

(A) Hypoxia

The effect of five percent oxygen (15 minutes) or 7.5, 8.5, 10 or 12 percent oxygen (30 minutes) on PGOT, PCPK, PLDH and PLDH isoenzyme ratio is shown in Figures 1 to 4 as well as in Tables 1 to 4. Data from control animals are included in these Figures and Tables. There was no change in plasma enzyme activity in the control animals indicating that surgical trauma or the prolonged anesthesia were not affecting the enzyme levels. In the experimental groups, generally, the plasma enzyme increase became progressively smaller as the degree of hypoxia decreased.

PGOT activity increased only in those animals ventilated with five or 7.5 percent oxygen. Therefore, the threshold for PGOT release must lie between 7.5 and 8.5 percent oxygen, which resulted in arterial oxygen tensions of 11 and 17 mmHg respectively (Table 5). All levels of hypoxia except 12 percent oxygen caused an increase in PCPK and PLDH. PCPK levels showed a progressive increase as the degree of the hypoxia increased. On the other hand, PLDH showed a large response to five percent oxygen, but the responses to 7.5, 8.5 and 10 percent oxygen were similar in magnitude. The threshold for

PCPK and PLDH release is between 10 and 12 percent oxygen, which produced arterial oxygen tensions of 24 and 31 mmHg respectively. Thus, it appears that CPK and LDH are released into the blood at similar levels of hypoxia and both are released with less severe levels of hypoxia than GOT.

The increase in PGOT activity was usually associated with ischemic ECG changes. Ischemic ECG changes were observed in all of the animals ventilated with five and 7.5 percent oxygen and in only two of the six animals ventilated with 8.5 percent oxygen. One of the animals ventilated with 10 percent oxygen and none of the animals ventilated with 12 percent oxygen showed ischemic ECG changes. Thus, it appears that the increase in PGOT activity correlates with ischemic ECG changes while increases in PCPK and PLDH are not necessarily associated with ischemic ECG changes.

This study was designed such that blood carbon dioxide changes would be negligible. The data indicates that there were only slight changes in the carbon dioxide tensions of arterial and venous blood (Tables 7 and 8). The rather high carbon dioxide tensions before hypoxia in the five and ten percent oxygen groups and in the control animals is due to the fact that in the initial stages of pentobarbital anesthesia respiration is depressed (Priano, Traber and Wilson, 1969). Because of this problem, the remainder of the groups were artificially ventilated with room air for about 15 minutes before being

ventilated with one of the various gas mixtures.

The arterial blood pressure increased to a peak within four to 10 minutes after the beginning of the hypoxia (Tables 9 and 10). The magnitude of the increase in blood pressure increased and the length of time required to reach the peak blood pressure decreased as the amount of oxygen in the inspired air decreased. The early increase in blood pressure was followed by a decrease to a steady state level which was below control measurements (except for the 12 percent oxygen group). The magnitude of the decrease in blood pressure seen at the end of the hypoxia increased as the amount of oxygen in the inspired air decreased.

There were only slight changes in heart rate during and following the various levels of hypoxia (Table 11). The one exception to this is the decrease in heart rate seen at the peak blood pressure in the group ventilated with five percent oxygen. This decrease in heart rate is probably due to baroreceptor stimulation (Nahas, 1956; Korner, 1959).

FIGURE 1

PGOT Response to Various Levels of Hypoxia.

Control ———

12 Percent Oxygen - - - - -

10 Percent Oxygen -·-·-

8.5 Percent Oxygen — — —

7.5 Percent Oxygen .....

5 Percent Oxygen -.-.-.

Shaded area represents the duration of hypoxia.

Standard error of the mean is not included for the 12, 10 and 8.5 percent oxygen groups.

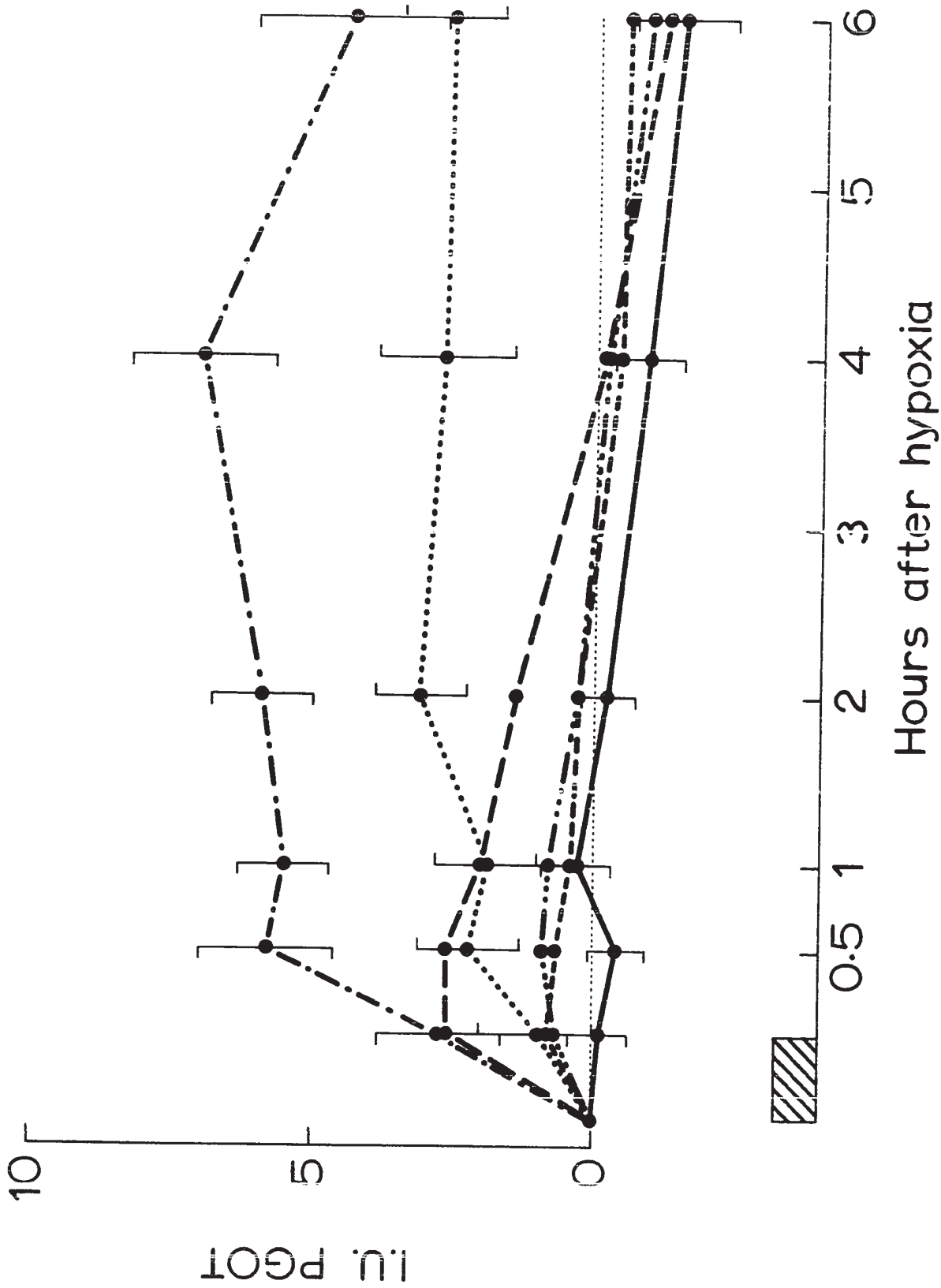
10
5
0
I.U. PGOT
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 2

PCPK Response to Various Levels of Hypoxia.

Control ——————

12 Percent Oxygen - - - - -

10 Percent Oxygen -...-...

8.5 Percent Oxygen — — —

7.5 Percent Oxygen .....

5 Percent Oxygen -.-.-.

Shaded area represents the duration of hypoxia.

Standard error of the mean is not included for the 12 and 7.5 percent oxygen groups.

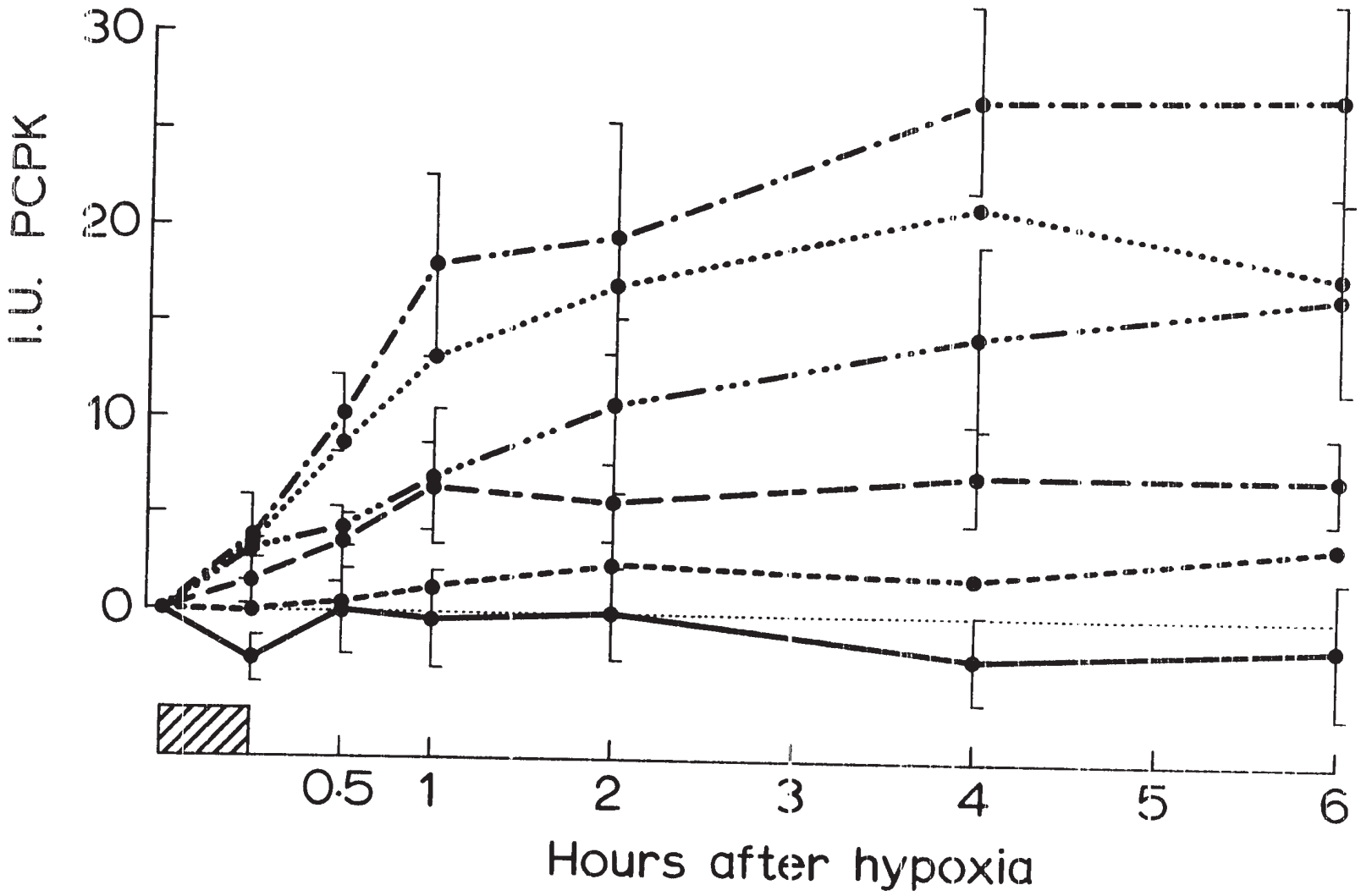
30
20
10
0
I.U. PCPK
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 3

PLDH Response to Various Levels of Hypoxia.

Control ———

12 Percent Oxygen - - - - -

10 Percent Oxygen - . - . - .

8.5 Percent Oxygen — — —

7.5 Percent Oxygen . . . . .

5 Percent Oxygen - - - - -

Shaded area represents the duration of hypoxia.

Standard error of the mean is not included for the 12, 10 and 8.5 percent oxygen groups.

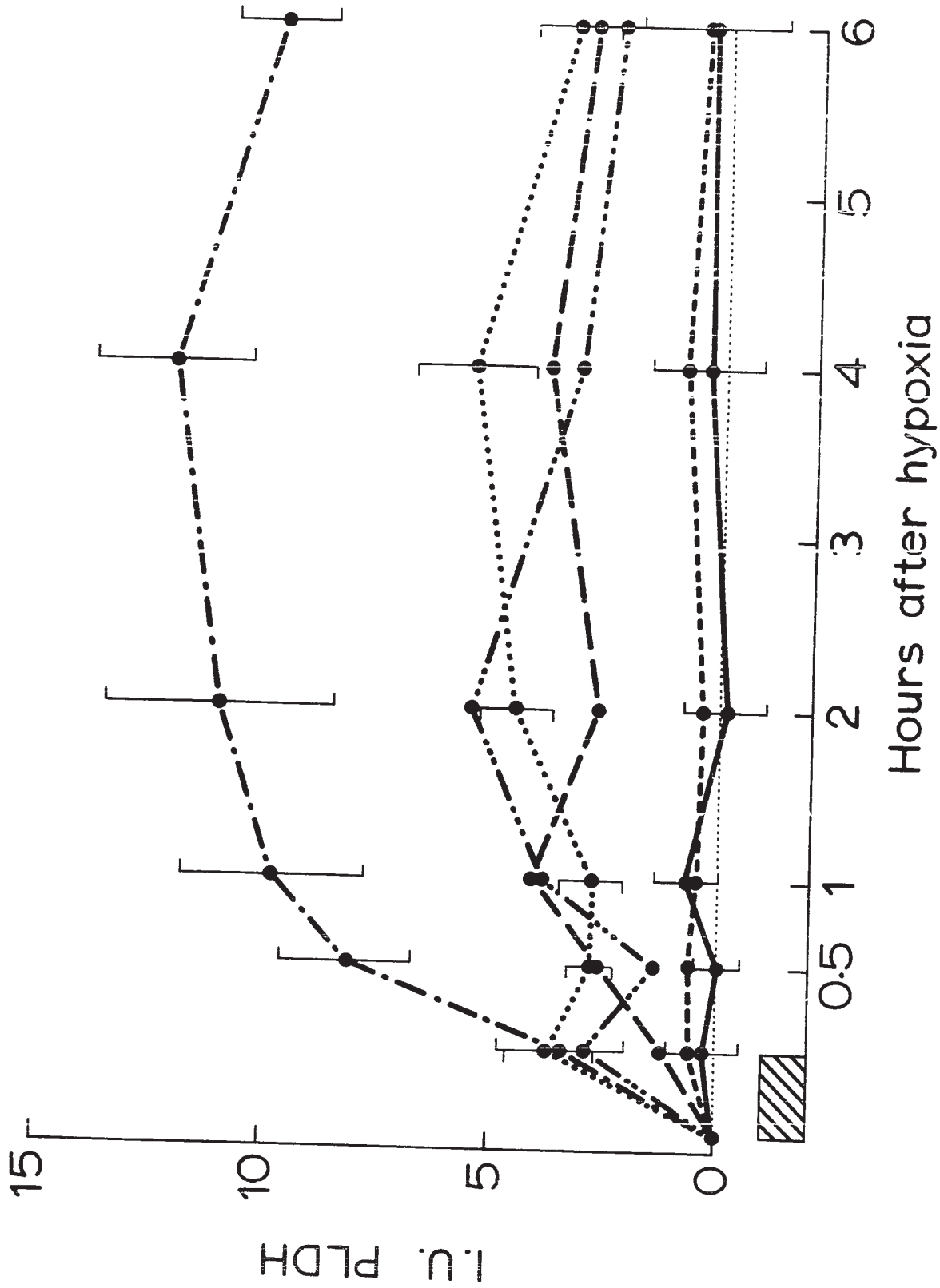
I.U. PLDH
15
10
5
0
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 4

PLDH Isoenzyme Ratio Response to Various Levels of Hypoxia.

Control ———

12 Percent Oxygen - - - - -

10 Percent Oxygen - . - . - . -

8.5 Percent Oxygen — — —

7.5 Percent Oxygen . . . . .

5 Percent Oxygen - - - - -

Shaded area represents the duration of hypoxia.

Standard error of the mean is not included for the 12, 8.5, 7.5 and 5 percent oxygen groups.

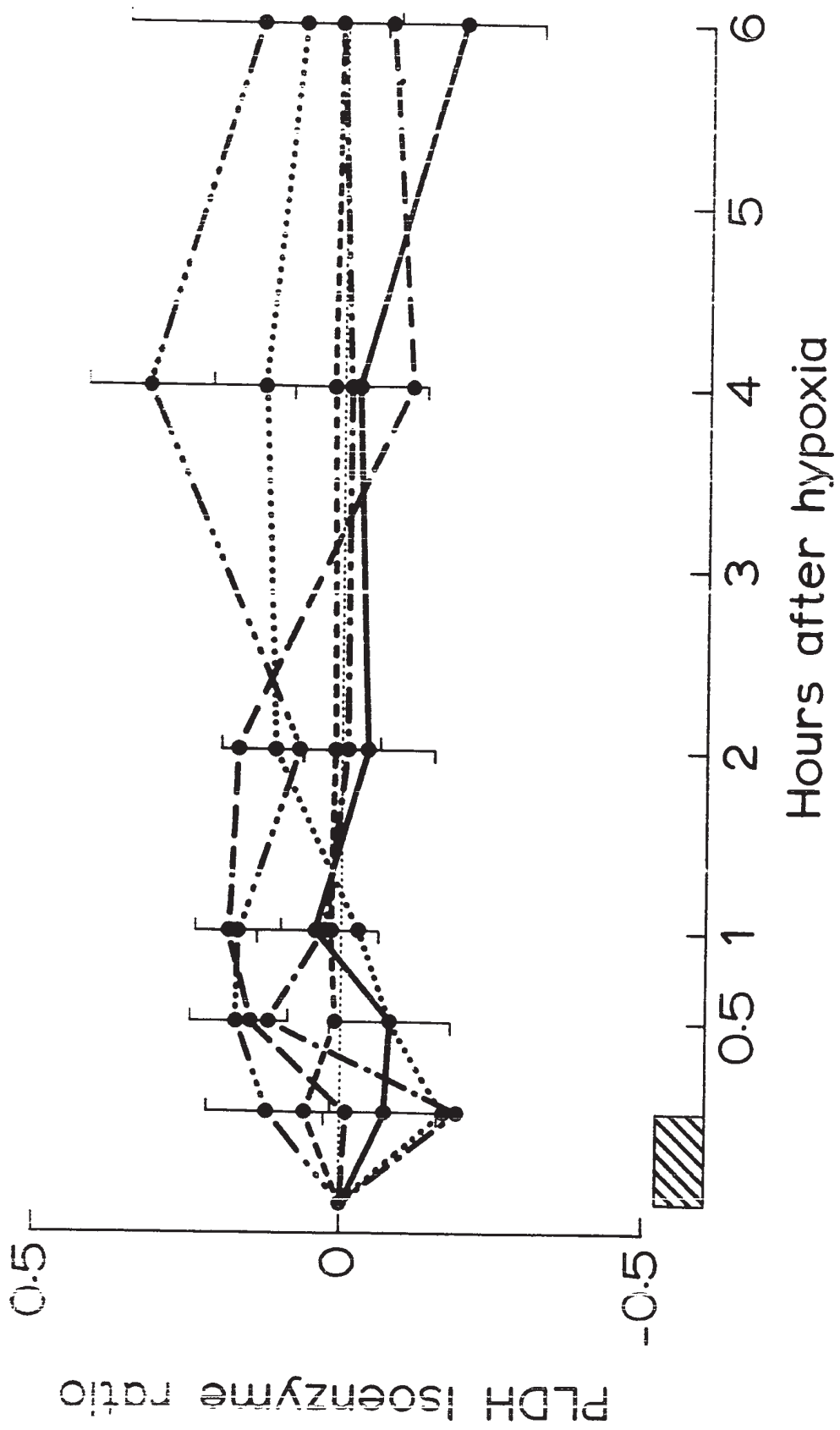
PLDH isoenzyme ratio
0.5
0
-0.5
0.5
1
2
3
4
5
6
Hours after hypoxia

TABLE 1

PGOT RESPONSE TO VARIOUS LEVELS OF HYPOXIA

Time	Control (30 min.)	12 Percent Oxygen (30 min.)	10 Percent Oxygen (30 min.)	8.5 Percent Oxygen (30 min.)	7.5 Percent Oxygen (30 min.)	5 Percent Oxygen (15 min.)
Before Hypoxia	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
End of Hypoxia	-0.1 ± 0.5*	0.8 ± 0.3	0.7 ± 0.5	2.6 ± 0.9	0.9 ± 1.0**	2.7 ± 1.1**
1/2 Hour after Hypoxia	-0.4 ± 0.5	0.7 ± 0.8	0.9 ± 0.5	2.6 ± 1.1	2.2 ± 0.9	5.8 ± 1.2
1 Hour	0.3 ± 0.6	0.4 ± 0.6	0.8 ± 0.2	2.0 ± 1.3	1.9 ± 0.9	5.5 ± 0.8
2 Hours	-0.2 ± 0.5	0.3 ± 0.6	0.3 ± 0.3	1.4 ± 1.6	3.1 ± 0.8	5.9 ± 0.9
4 Hours	-0.9 ± 0.6	-0.4 ± 0.4	-0.2 ± 0.4	-0.2 ± 1.5	2.7 ± 1.2	7.0 ± 1.3
6 Hours	-1.5 ± 0.9	-0.5 ± 0.7	-0.9 ± 0.6	-1.2 ± 0.7	2.6 ± 0.9	4.4 ± 1.7

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.
** P is less than 0.05.

TABLE 2

PCPK RESPONSE TO VARIOUS LEVELS OF HYPOXIA

Time	Control (30 min.)	12 Percent Oxygen (30 min.)	10 Percent Oxygen (30 min.)	8.5 Percent Oxygen (30 min.)	7.5 Percent Oxygen (30 min.)	5 Percent Oxygen (15 min.)
Before Hypoxia	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
End of Hypoxia	-2.5 ± 1.1*	00.0 ± 0.7	3.2 ± 0.6 **	1.7 ± 1.5 **	3.5 ± 1.3**	3.7 ± 2.3**
1/2 Hour after Hypoxia	00.0 ± 2.2	0.2 ± 0.6	4.7 ± 0.6	3.7 ± 2.1	8.7 ± 1.8	10.3 ± 2.0
1 Hour	-0.3 ± 2.5	1.2 ± 1.1	7.0 ± 3.5	6.8 ± 2.3	13.0 ± 3.2	18.0 ± 4.6
2 Hours	00.0 ± 2.4	2.5 ± 0.6	10.8 ± 4.5	5.8 ± 1.9	17.0 ± 4.1	19.5 ± 5.9
4 Hours	-2.2 ± 2.4	2.0 ± 0.7	14.5 ± 4.8	7.3 ± 2.7	21.3 ± 6.4	26.7 ± 4.8
6 Hours	-1.5 ± 3.6	3.7 ± 1.1	16.7 ± 5.0	7.2 ± 2.3	17.8 ± 4.6	27.0 ± 5.1

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean
** P is less than 0.05.

TABLE 2

PCPK RESPONSE TO VARIOUS LEVELS OF HYPOXIA

Time	Control (30 min.)	12 Percent Oxygen (30 min.)	10 Percent Oxygen (30 min.)	8.5 Percent Oxygen (30 min.)	7.5 Percent Oxygen (30 min.)	5 Percent Oxygen (15 min.)
Before Hypoxia	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
End of Hypoxia	-2.5 ± 1.1*	00.0 ± 0.7	3.2 ± 0.6**	1.7 ± 1.5**	3.5 ± 1.3**	3.7 ± 2.3**
1/2 Hour after Hypoxia	00.0 ± 2.2	0.2 ± 0.6	4.7 ± 0.6	3.7 ± 2.1	8.7 ± 1.8	10.3 ± 2.0
1 Hour	-0.3 ± 2.5	1.2 ± 1.1	7.0 ± 3.5	6.8 ± 2.3	13.0 ± 3.2	18.0 ± 4.6
2 Hours	00.0 ± 2.4	2.5 ± 0.6	10.8 ± 4.5	5.8 ± 1.9	17.0 ± 4.1	19.5 ± 5.9
4 Hours	-2.2 ± 2.4	2.0 ± 0.7	14.5 ± 4.8	7.3 ± 2.7	21.3 ± 6.4	26.7 ± 4.8
6 Hours	-1.5 ± 3.6	3.7 ± 1.1	16.7 ± 5.0	7.2 ± 2.3	17.8 ± 4.6	27.0 ± 5.1

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean
** P is less than 0.05.

TABLE 3

PLDH RESPONSE TO VARIOUS LEVELS OF HYPOXIA

Time	Control (30 min.)	12 Percent Oxygen (30 min.)	10 Percent Oxygen (30 min.)	8.5 Percent Oxygen (30 min.)	7.5 Percent Oxygen (30 min.)	5 Percent Oxygen (15 min.)
Before Hypoxia	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
End of Hypoxia	0.3 ± 0.8*	0.6 ± 0.5	2.9 ± 1.0**	1.2 ± 0.6**	3.8 ± 0.9**	3.4 ± 1.4**
1/2 Hour After Hypoxia	00.0 ± 0.6	0.6 ± 0.5	1.4 ± 0.5	2.6 ± 1.2	2.8 ± 0.5	8.1 ± 1.5
1 Hour	0.7 ± 0.7	0.5 ± 0.4	3.9 ± 1.0	4.1 ± 1.1	2.8 ± 0.7	9.8 ± 2.0
2 Hours	-0.1 ± 0.9	0.4 ± 0.5	5.5 ± 1.4	2.7 ± 1.2	4.5 ± 0.8	11.0 ± 2.5
4 Hours	0.4 ± 1.2	0.9 ± 0.4	3.2 ± 1.7	3.9 ± 1.2	5.5 ± 1.3	12.1 ± 1.6
6 Hours	0.4 ± 1.6	0.5 ± 0.7	2.4 ± 1.5	3.0 ± 1.3	3.4 ± 1.0	9.8 ± 1.1

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.
** P is less than 0.05.

TABLE 4

PLDH ISOENZYME RATIO RESPONSE TO VARIOUS LEVELS OF HYPOXIA

Time	Control (30 min.)	12 Percent Oxygen (30 min.)	10 Percent Oxygen (30 min.)	8.5 Percent Oxygen (30 min.)	7.5 Percent Oxygen (30 min.)	5 Percent Oxygen (15 min.)
Before Hypoxia	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
End of Hypoxia	-0.07 ± 0.09*	0.06 ± 0.04	0.12 ± 0,10	-0.01 ± 0.09	-0.17 ± 0.08	-0.19 ± 0.13
1/2 Hour after Hypoxia	-0.08 ± 0.10	0.01 ± 0.06	0.17 ± 0.08	0.15 ± 0.11	-0.08 ± 0.07	0.12 ± 0.11
1 Hour	0.04 ± 0.10	0.02 ± 0.07	0.17 ± 0.07	0.18 ± 0.09	-0.03 ± 0.10	0.03 ± 0.15
2 Hours	-0.04 ± 0.11	0.01 ± 0.05	0.07 ± 0.13	0.17 ± 0.10	0.11 ± 0.14	-0.01 ± 0.20
4 Hours	-0.02 ± 0.11	0.02 ± 0.08	0.32 ± 0.10	-0.11 ± 0.24	0.13 ± 0.06	-0.01 ± 0.23
6 Hours	-0.19 ± 0.13	0.01 ± 0.10	0.14 ± 0.22	-0.07 ± 0.15	0.07 ± 0.14	0.01 ± 0.06

n is equal to 6.

* Standard Error of the Mean

TABLE 5

ARTERIAL BLOOD OXYGEN TENSION RESPONSE TO VARIOUS LEVELS OF HYPOXIA

Time	Control (30 min.)	12 Percent Oxygen (30 min.)	10 Percent Oxygen (30 min.)	8.5 Percent Oxygen (30 min.)	7.5 Percent Oxygen (30 min.)	5 Percent Oxygen (15 min.)
Before Hypoxia	69 ± 7*	67 ± 2	61 ± 3	73 ± 2	72 ± 2	60 ± 4
End of Hypoxia	75 ± 4	31 ± 1	24 ± 2	17 ± 2	11 ± 1	11 ± 2
1/2 Hour after Hypoxia	87 ± 5	72 ± 3	74 ± 7	71 ± 4	71 ± 3	70 ± 4
1 Hour	85 ± 2	73 ± 1	83 ± 7	73 ± 6	73 ± 3	82 ± 4
2 Hours	87 ± 3	67 ± 2	84 ± 9	76 ± 6	73 ± 3	85 ± 3
4 Hours	84 ± 4	66 ± 3	85 ± 12	71 ± 6	71 ± 4	85 ± 7
6 Hours	82 ± 4	68 ± 3	80 ± 9	69 ± 4	69 ± 4	87 ± 4

n is equal to 6.
Oxygen tension is expressed as mmHg.
* Standard Error of the Mean.

TABLE 6

VENOUS BLOOD OXYGEN TENSION RESPONSE TO VARIOUS LEVELS OF HYPOXIA

Time	Control (30 min.)	12 Percent Oxygen (30 min.)	10 Percent Oxygen (30 min.)	8.5 Percent Oxygen (30 min.)	7.5 Percent Oxygen (30 min.)	5 Percent Oxygen (15 min.)
Before Hypoxia	47 ± 2*	46 ± 1	44 ± 2	44 ± 1	43 ± 3	36 ± 3
End of Hypoxia	42 ± 2	23 ± 1	20 ± 1	9 ± 2	6 ± 1	4 ± 1
1/2 Hour after Hypoxia	45 ± 3	46 ± 2	47 ± 1	45 ± 3	46 ± 3	40 ± 3
1 Hour	46 ± 3	44 ± 2	49 ± 2	48 ± 3	44 ± 3	38 ± 4
2 Hours	45 ± 2	41 ± 2	47 ± 3	48 ± 2	41 ± 3	40 ± 3
4 Hours	44 ± 2	41 ± 2	45 ± 2	44 ± 2	39 ± 2	37 ± 4
6 Hours	42 ± 1	40 ± 1	42 ± 2	42 ± 2	41 ± 2	38 ± 3

n is equal to 6.
Oxygen tension is expressed as mmHg.
* Standard Error of the Mean.

TABLE 7

ARTERIAL BLOOD CARBON DIOXIDE TENSION RESPONSE TO VARIOUS LEVELS OF HYPOXIA

Time	Control (30 min.)	12 Percent Oxygen (30 min.)	10 Percent Oxygen (30 min.)	8.5 Percent Oxygen (30 min.)	7.5 Percent Oxygen (30 min.)	5 Percent Oxygen (15 min.)
Before Hypoxia	44 ± 2*	35 ± 1	56 ± 2	35 ± 1	30 ± 2	44 ± 5
End of Hypoxia	32 ± 2	33 ± 2	41 ± 2	39 ± 2	32 ± 2	32 ± 2
1/2 Hour after Hypoxia	29 ± 2	35 ± 1	38 ± 5	39 ± 3	33 ± 1	33 ± 2
1 Hour	31 ± 1	34 ± 1	37 ± 4	36 ± 2	32 ± 1	31 ± 2
2 Hours	33 ± 2	37 ± 1	46 ± 4	36 ± 2	31 ± 1	33 ± 2
4 Hours	35 ± 3	37 ± 2	49 ± 5	35 ± 2	31 ± 2	33 ± 1
6 Hours	32 ± 4	35 ± 1	46 ± 5	33 ± 2	31 ± 2	32 ± 2

n is equal to 6.
Carbon dioxide tension is expressed as mmHg.
* Standard Error of the Mean.

TABLE 8

VENOUS BLOOD CARBON DIOXIDE TENSION RESPONSE TO VARIOUS LEVELS OF HYPOXIA

Time	Control (30 min.)	12 Percent Oxygen (30 min.)	10 Percent Oxygen (30 min.)	8.5 Percent Oxygen (30 min.)	7.5 Percent Oxygen (30 min.)	5 Percent Oxygen (15 min.)
Before Hypoxia	45 ± 2*	36 ± 1	54 ± 4	34 ± 2	34 ± 2	46 ± 5
End of Hypoxia	35 ± 3	36 ± 1	43 ± 2	41 ± 2	36 ± 4	38 ± 2
1/2 Hour after Hypoxia	36 ± 3	37 ± 1	41 ± 5	42 ± 3	36 ± 1	38 ± 3
1 Hour	38 ± 2	38 ± 1	42 ± 5	39 ± 2	35 ± 1	36 ± 3
2 Hours	39 ± 2	40 ± 2	48 ± 4	39 ± 2	34 ± 1	37 ± 3
4 Hours	43 ± 4	41 ± 2	51 ± 5	37 ± 2	34 ± 3	42 ± 5
6 Hours	40 ± 4	40 ± 1	48 ± 5	37 ± 3	32 ± 2	37 ± 3

n is equal to 6.
Carbon dioxide tension is expressed as mmHg.
* Standard Error of the Mean.

TABLE 9

ARTERIAL SYSTOLIC BLOOD PRESSURE RESPONSE TO VARIOUS LEVELS OF HYPOXIA

Time	Control (30 min.)	12 Percent Oxygen (30 min.)	10 Percent Oxygen (30 min.)	8.5 Percent Oxygen (30 min.)	7.5 Percent Oxygen (30 min.)	5 Percent Oxygen (15 min.)
Before Hypoxia	141 ± 10*	149 ± 3	134 ± 8	144 ± 8	151 ± 5	140 ± 3
Peak Blood Pressure		178 ± 7 (6 min.)***	161 ± 8 (10 min.)	191 ± 10 (7 min.)	219 ± 5 (5 min.)	180 ± 7 (4 min.)
End of Hypoxia	150 ± 14	153 ± 6	129 ± 16	140 ± 19	122 ± 13	84 ± 17
1/2 Hour after Hypoxia	154 ± 13	146 ± 5	125 ± 13	145 ± 11	138 ± 5	122 ± 4
1 Hour	156 ± 9	142 ± 2	128 ± 10	152 ± 8	135 ± 4	129 ± 4
2 Hours	158 ± 9	143 ± 3	137 ± 10	153 ± 8	136 ± 6	131 ± 5
4 Hours	167 ± 11	143 ± 4	142 ± 9	157 ± 8	141 ± 6	139 ± 6
6 Hours	164 ± 8	147 ± 4	146 ± 12	153 ± 8	143 ± 5	134 ± 7

n is equal to 6.
Blood pressure is expressed as mmHg.
* Standard Error of the Mean.
*** Time after the beginning of hypoxia when peak blood pressure was reached.

TABLE 10

ARTERIAL DIASTOLIC BLOOD PRESSURE RESPONSE TO VARIOUS LEVELS OF HYPOXIA

Time	Control (30 min.)	12 Percent Oxygen (30 min.)	10 Percent Oxygen (30 min.)	8.5 Percent Oxygen (30 min.)	7.5 Percent Oxygen (30 min.)	5 Percent Oxygen (15 min.)
Before Hypoxia	117 ± 9*	123 ± 3	108 ± 7	120 ± 6	123 ± 4	118 ± 7
Peak Blood Pressure		144 ± 5 (6 min.)***	127 ± 10 (10 min.)	151 ± 8 (7 min.)	165 ± 5 (5 min.)	138 ± 7 (4 min.)
End of Hypoxia	123 ± 13	127 ± 5	108 ± 14	108 ± 14	94 ± 14	69 ± 14
1/2 Hour after Hypoxia	127 ± 10	121 ± 4	105 ± 10	116 ± 7	115 ± 11	110 ± 3
1 Hour	130 ± 9	118 ± 3	106 ± 10	113 ± 5	110 ± 3	104 ± 2
2 Hours	131 ± 9	118 ± 3	116 ± 9	118 ± 5	109 ± 5	108 ± 4
4 Hours	136 ± 9	118 ± 5	117 ± 9	123 ± 5	113 ± 5	117 ± 6
6 Hours	136 ± 8	119 ± 4	122 ± 10	121 ± 6	116 ± 3	114 ± 8

n is equal to 6.

* Standard Error of the Mean.

*** Time after the beginning of hypoxia when peak blood pressure was reached.

Blood pressure is expressed as mmHg.

TABLE 11

HEART RATE RESPONSE TO VARIOUS LEVELS OF HYPOXIA

Time	Control (30 min.)	12 Percent Oxygen (30 min.)	10 Percent Oxygen (30 min.)	8.5 Percent Oxygen (30 min.)	7.5 Percent Oxygen (30 min.)	5 Percent Oxygen (15 min.)
Before Hypoxia	182 ± 13*	190 ± 12	173 ± 10	156 ± 12	175 ± 10	179 ± 8
Heart Rate at Peak Blood Pressure		189 ± 13 (6 min.)***	174 ± 14 (10 min.)	155 ± 10 (7 min.)	172 ± 10 (5 min.)	137 ± 9 (4 min.)
End of Hypoxia	156 ± 12	179 ± 16	168 ± 13	136 ± 14	171 ± 12	168 ± 7
1/2 Hour after Hypoxia	157 ± 11	186 ± 12	171 ± 10	154 ± 12	171 ± 5	184 ± 6
1 Hour	158 ± 14	183 ± 10	167 ± 8	159 ± 15	170 ± 9	184 ± 6
2 Hours	150 ± 11	182 ± 14	163 ± 7	158 ± 14	159 ± 12	179 ± 9
4 Hours	149 ± 13	177 ± 14	152 ± 12	146 ± 12	153 ± 9	167 ± 17
6 Hours	162 ± 15	174 ± 14	159 ± 14	146 ± 16	171 ± 9	173 ± 17

n is equal to 6.
Heart rate is expressed as beats per minute.
* Standard Error of the Mean.
*** Time after the beginning of hypoxia when peak blood pressure was reached.

(B) Simulated Exercise

The plasma enzyme response to simulated exercise was quite different from the response to hypoxia (Figures 5 to 8; Tables 12 and 13). None of the enzymes studied showed an increase following the five pulses per second stimulation. There was an increase in PGOT and PLDH activity following the 10 pulses per second stimulation. There was no change in PCPK activity in either group. The magnitude of the PGOT increase was similar to that seen following 7.5 percent oxygen, however, the peak increase occurred sooner and there was a return to control values at four and six hours after the simulated exercise. The pattern of the PLDH response was similar to the PGOT response but the peak increase was greater (comparable to the response to five percent oxygen). There was no change in the PLDH isoenzyme ratio.

Oxygen consumption measured in four dogs during muscle stimulation at five pulses per second was 17.5 ± 3.2 milliliters of oxygen per kilogram per minute. Thirty minutes after stimulation it was 8.0 ± 2.3 milliliters of oxygen per kilogram per minute and two hours after stimulation it was 5.7 ± 0.6 milliliters of oxygen per kilogram per minute. The oxygen consumption during the 10 pulses per second stimulation was measured in all six animals and was 21.0 ± 2.2 milliliters of oxygen per kilogram per minute. Thirty minutes and two hours after the stimulation it was 5.6 ± 2.5 milliliters of

oxygen per kilogram per minute. The oxygen consumption of these two groups during simulated exercise is not significantly different.

Changes in arterial and venous oxygen and carbon dioxide tensions were quite similar for both of these groups (Tables 14 and 15). There was a slight increase in arterial and venous carbon dioxide tensions and a slight decrease in arterial and venous oxygen tensions at the end of the stimulation. These changes may have been due to the respiratory depression caused by the anesthetic since these animals were not artificially respired during the stimulation. There was a decrease in oxygen tension in the femoral vein (Table 14) and both groups reached the same oxygen tensions at the end of the stimulation (22 mmHg). Carbon dioxide tension in the femoral vein increased in both groups (Table 15). There was a greater increase following the five pulses per second stimulation (62 mmHg) than there was following the 10 pulses per second stimulation (46 mmHg).

There was a slight decrease in systolic blood pressure with five pulses per second stimulation (Table 16) and a slight increase in pulse pressure. There was no change in the systolic pressure during the 10 pulses per second stimulation, but there was a slight increase in pulse pressure. Stimulation at five pulses per second caused no change in heart rate, but stimulation at 10 pulses per second caused the heart rate to increase from 159 to 175 beats per minute (Table 16).

FIGURE 5

PGOT Response to Simulated Exercise.

5 Pulses per second stimulation — — —

10 Pulses per second stimulation .....

7.5 Percent Oxygen .....

Control ———

Shaded area represents the duration of stimulation or hypoxia.

Standard Error of the Mean is not included for the 7.5 percent oxygen group.

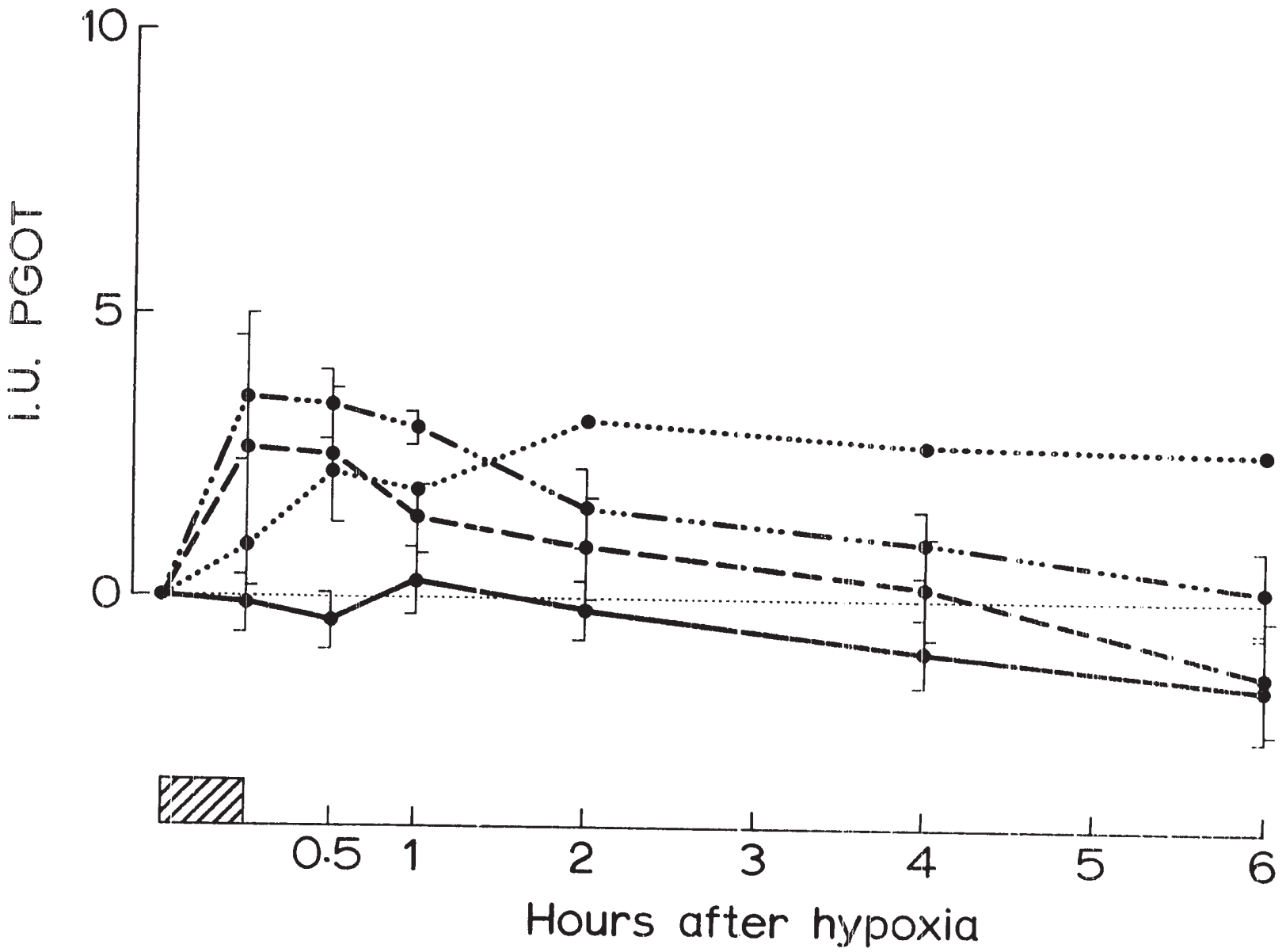
10
5
0
I.U. PGOT
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 6

PCPK Response to Simulated Exercise.

5 Pulses per second stimulation — — —

10 Pulses per second stimulation -.-.-.-

7.5 Percent oxygen .....

Control _____

Shaded area represents the duration of stimulation or hypoxia.

Standard Error of the Mean is not included for the 5 pulses per second stimulation group.

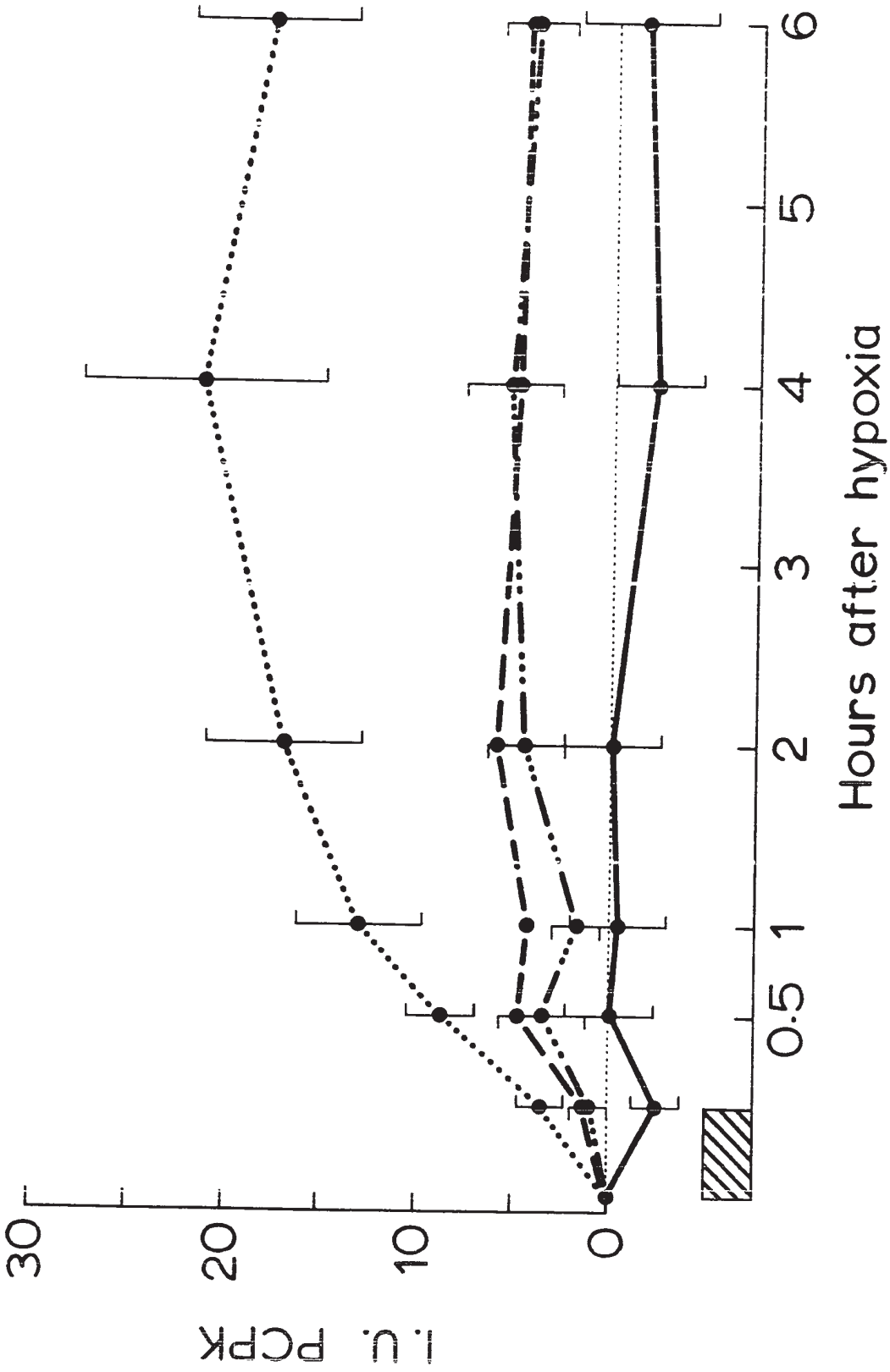
I. U. PCPK
30
20
10
0
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 7

PLDH Response to Simulated Exercise.

5 Pulses per second stimulation — — —

10 Pulses per second stimulation -.-.-.-

5 Percent oxygen - - - - -

7.5 Percent Oxygen .....

Control _____

Shaded area represents the duration of stimulation or hypoxia.

Standard Error of the Mean is not included for the 7.5 percent oxygen group.

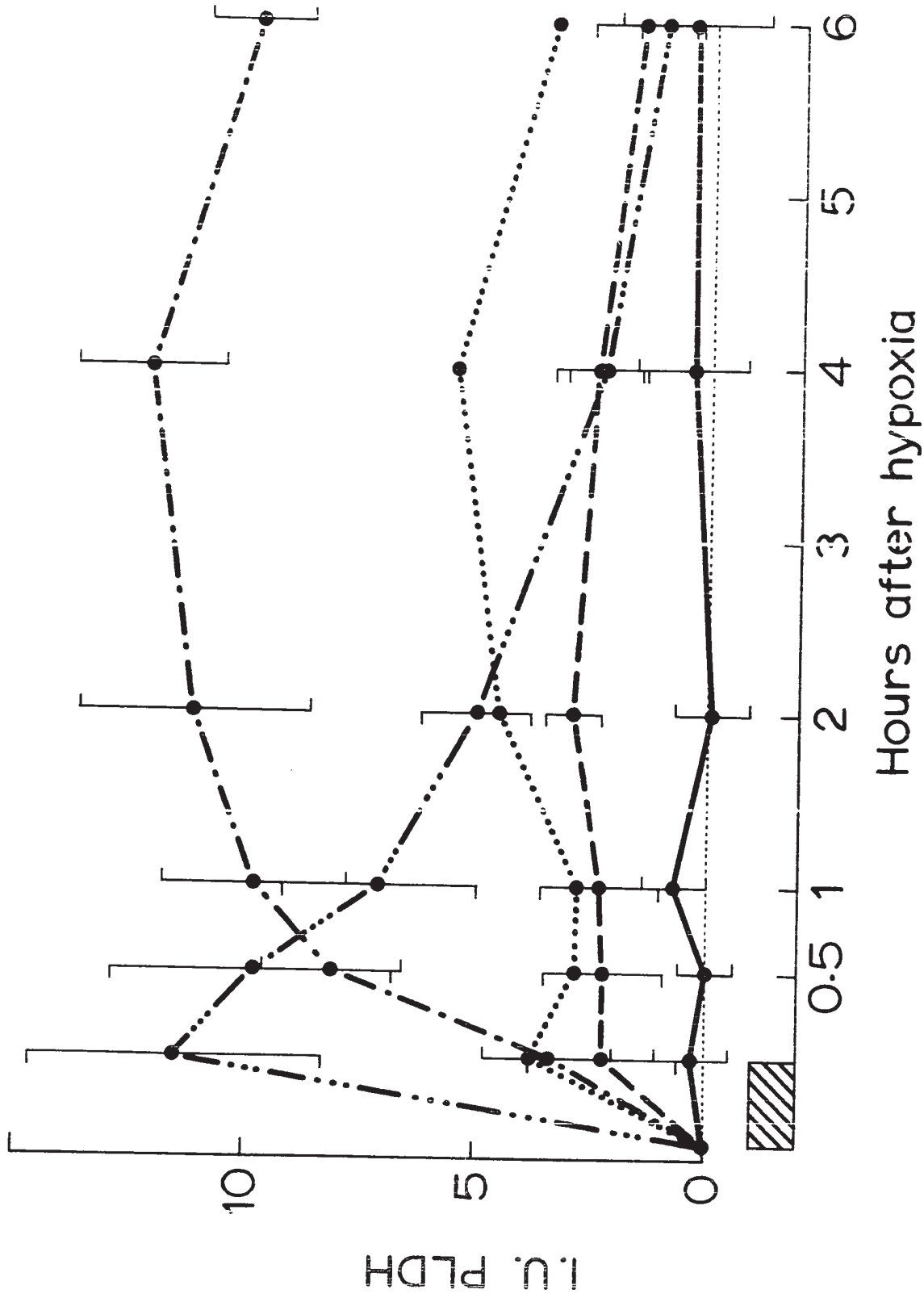
I.U. PLDH
0
5
10
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 8

PLDH Isoenzyme Ratio Response to Simulated Exercise.

5 Pulses per second stimulation — — —

10 Pulses per second stimulation -·-·-·-

Control ————

Shaded area represents the duration of stimulation.

Standard Error of the Mean is not included for the 5 pulses per second stimulation group.

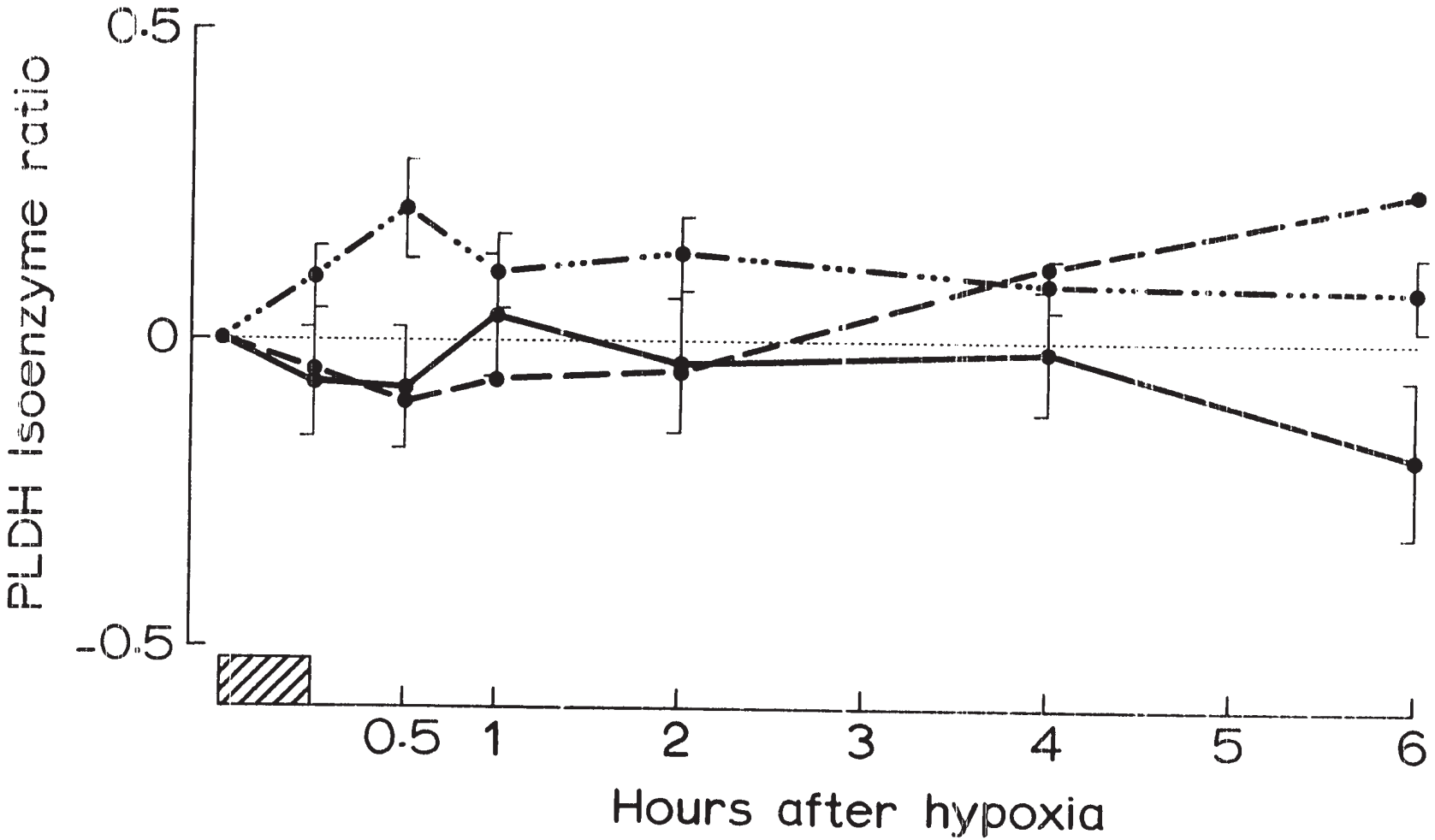
0.5
0
-0.5
PLDH Isoenzyme ratio
0.5
1
2
3
4
5
6
Hours after hypoxia

TABLE 12

PGOT AND PCPK RESPONSE TO SIMULATED EXERCISE

Time	PGOT		PCPK	
	5 pulses per sec. (30 minutes)	10 pulses per sec. (30 minutes)	5 pulses per sec. (30 minutes)	10 pulses per sec. (30 minutes)
Before Stimulation	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
End of Stimulation	2.6 ± 2.4 *	3.5 ± 1.1**	1.5 ± 1.5	1.0 ± 1.0
1/2 Hour After	2.5 ± 1.4	3.4 ± 0.6	4.8 ± 2.1	3.5 ± 2.3
1 Hour	1.4 ± 0.6	3.0 ± 0.3	4.3 ± 4.3	1.8 ± 1.1
2 Hours	0.9 ± 0.9	1.6 ± 0.7	6.0 ± 6.1	4.5 ± 2.0
4 Hours	0.2 ± 0.8	0.1 ± 0.6	5.0 ± 5.6	5.3 ± 2.3
6 Hours	-1.3 ± 1.0	0.2 ± 0.7	4.5 ± 3.0	4.3 ± 1.8

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.
** P is less than 0.05.

TABLE 13

PLDH AND PLDH ISOENZYME RATIO RESPONSE TO SIMULATED EXERCISE

Time	PLDH		PLDH ISOENZYME RATIO	
	5 pulses per sec. (30 minutes)	10 pulses per sec. (30 minutes)	5 pulses per sec. (30 minutes)	10 pulses per sec. (30 minutes)
Before Stimulation	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
End of Stimulation	2.2 ± 1.6*	11.5 ± 3.2**	-0.05 ± 0.06	0.10 ± 0.05
1/2 Hour After	2.2 ± 1.3	9.8 ± 3.0	-0.10 ± 0.06	0.21 ± 0.08
1 Hour	2.3 ± 1.3	7.1 ± 2.1	-0.06 ± 0.06	0.11 ± 0.06
2 Hours	2.9 ± 0.6	5.0 ± 1.2	-0.05 ± 0.10	0.14 ± 0.06
4 Hours	2.4 ± 1.0	2.3 ± 0.8	0.12 ± 0.16	0.09 ± 0.03
6 Hours	1.5 ± 1.1	1.0 ± 0.6	0.24 ± 0.14	0.08 ± 0.06

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.
** P is less than 0.05.

TABLE 14

ARTERIAL, RIGHT ATRIAL AND FEMORAL VEIN BLOOD OXYGEN TENSION RESPONSE TO SIMULATED EXERCISE

Time	Arterial		Right Atrial		Femoral Vein	
	5 pulses/ sec.	10 pulses/ sec.	5 pulses/ sec.	10 pulses/ sec.	5 pulses/ sec.	10 pulses/ sec.
Before Stimulation	75 ± 2*	73 ± 2	42 ± 4	47 ± 2	47 ± 5	45 ± 3
End of Stimulation	56 ± 2	60 ± 2	32 ± 3	35 ± 2	22 ± 3	22 ± 1
1/2 Hour After	64 ± 3	65 ± 3	39 ± 3	39 ± 1	45 ± 2	40 ± 2
1 Hour	68 ± 3	68 ± 4	40 ± 3	41 ± 2	43 ± 4	42 ± 2
2 Hours	68 ± 3	68 ± 4	41 ± 3	40 ± 1	44 ± 4	37 ± 2
4 Hours	65 ± 2	69 ± 4	40 ± 3	38 ± 1	38 ± 3	36 ± 2
6 Hours	63 ± 4	64 ± 2	41 ± 3	39 ± 2	40 ± 2	33 ± 2

n is equal to 6.
* Standard Error of the Mean
Oxygen tension is expressed as mmHg.

TABLE 15

ARTERIAL, RIGHT ATRIAL AND FEMORAL VEIN BLOOD CARBON DIOXIDE TENSION RESPONSE TO SIMULATED EXERCISE

Time	Arterial		Right Atrial		Femoral Vein	
	5 pulses/ sec.	10 pulses/ sec.	5 pulses/ sec.	10 pulses/ sec.	5 pulses/ sec.	10 pulses/ sec.
Before Stimulation	32 ± 1*	36 ± 2	37 ± 1	38 ± 2	36 ± 1	38 ± 2
End of Stimulation	43 ± 5	36 ± 3	49 ± 4	42 ± 3	62 ± 4	46 ± 3
1/2 Hour After	43 ± 4	36 ± 2	46 ± 4	38 ± 3	46 ± 3	39 ± 2
1 Hour	41 ± 4	37 ± 3	47 ± 5	40 ± 3	48 ± 4	39 ± 2
2 Hours	42 ± 5	38 ± 3	47 ± 5	41 ± 3	46 ± 3	41 ± 3
4 Hours	44 ± 6	37 ± 3	49 ± 6	42 ± 3	49 ± 6	43 ± 3
6 Hours	43 ± 5	39 ± 2	49 ± 6	44 ± 3	52 ± 5	45 ± 3

n is equal to 6.
Carbon Dioxide tension is expressed as mmHg.
* Standard Error of the Mean.

TABLE 16

ARTERIAL SYSTOLIC AND DIASTOLIC BLOOD PRESSURE AND HEART RATE RESPONSE TO SIMULATED EXERCISE

Time	Systolic		Diastolic		Heart Rate	
	5 pulses/ sec.	10 pulses/ sec.	5 pulses/ sec.	10 pulses/ sec.	5 pulses/ sec.	10 pulses/ sec.
Before Stimulation	131 ± 7*	138 ± 6	109 ± 6	113 ± 6	155 ± 13	159 ± 3
End of Stimulation	120 ± 10	136 ± 8	92 ± 9	103 ± 5	156 ± 11	175 ± 6
1/2 Hour After	133 ± 5	127 ± 3	106 ± 5	101 ± 3	138 ± 15	151 ± 9
1 Hour	133 ± 6	132 ± 5	106 ± 6	105 ± 6	139 ± 19	151 ± 8
2 Hours	137 ± 8	135 ± 7	112 ± 8	108 ± 6	144 ± 20	156 ± 7
4 Hours	136 ± 9	123 ± 7	113 ± 9	107 ± 8	141 ± 20	160 ± 10
6 Hours	137 ± 8	136 ± 7	113 ± 7	108 ± 7	136 ± 21	140 ± 15

n is equal to 6.
Blood Pressure is expressed as mmHg.
Heart Rate is expressed as beats per minute.
* Standard Error of the Mean.

(C) Multiple Sampling Sites

Enzyme analysis of blood samples taken simultaneously from four sampling sites was carried out on animals that were ventilated with five percent oxygen for 15 minutes. The four sampling sites were the carotid artery, right atrium, femoral vein and the inferior vena cava just above the opening of the hepatic veins. There was no difference in PGOT, PCPK, PLDH activity or PLDH isoenzyme ratio in blood samples taken from the different sampling sites (Figures 9 to 12; Tables 17 to 20).

Arterial and venous (right atrium) blood samples were analyzed for plasma enzyme activity in control animals and in animals ventilated with 10 percent oxygen for 30 minutes. There was no difference in plasma enzyme activity between arterial and venous samples in the control group (Figures 13 to 16; Tables 21 and 22). Similarly, there was no arterio-venous difference in PGOT, PCPK AND PLDH isoenzyme ratio in the 10 percent oxygen group (Figures 17, 18, and 20; Tables 23 and 24). In this group, the arterial PLDH activity was higher than the venous PLDH activity (Figure 19, Table 24).

FIGURE 9

PGOT activity of samples taken simultaneously from four different sites in animals respired with five percent oxygen for 15 minutes.

Carotid Artery ———

Right Atrium .....

Femoral Vein — — —

Inferior Vena Cava -...-...

Shaded area represents the duration of hypoxia.

Standard Error of the Mean is not included for the carotid artery and right atrium samples.

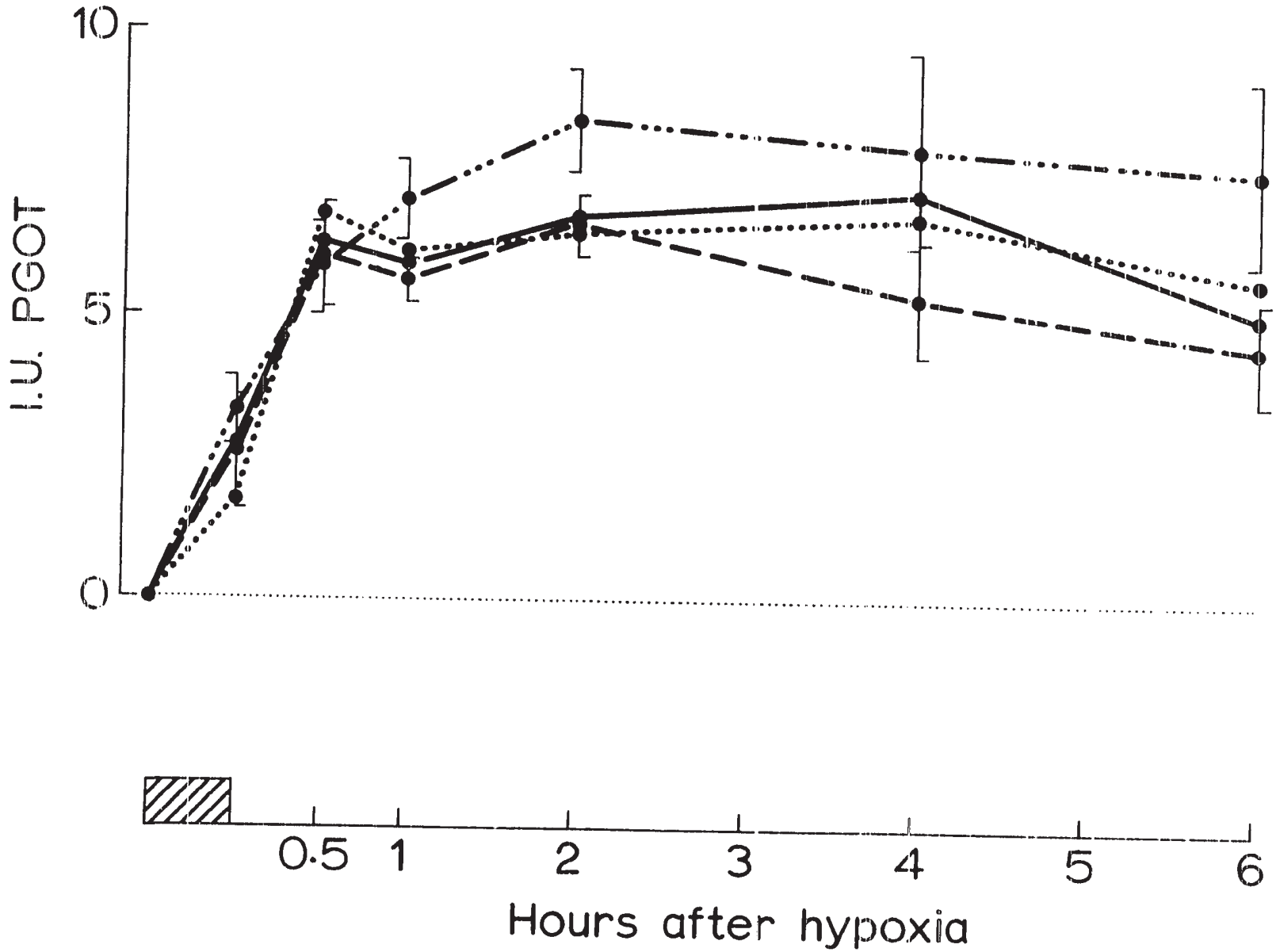
10
5
0
I.U. PGOT
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 10

PCPK activity of samples taken simultaneously from four different sampling sites in animals respired with five percent oxygen for 15 minutes.

Carotid Artery _____

Right Atrium .....

Femoral Vein — — —

Inferior Vena Cava -.-.-.-

Shaded area represents the duration of hypoxia.

Standard Error of the Mean is not included for the right atrium, femoral vein and inferior vena cava samples.

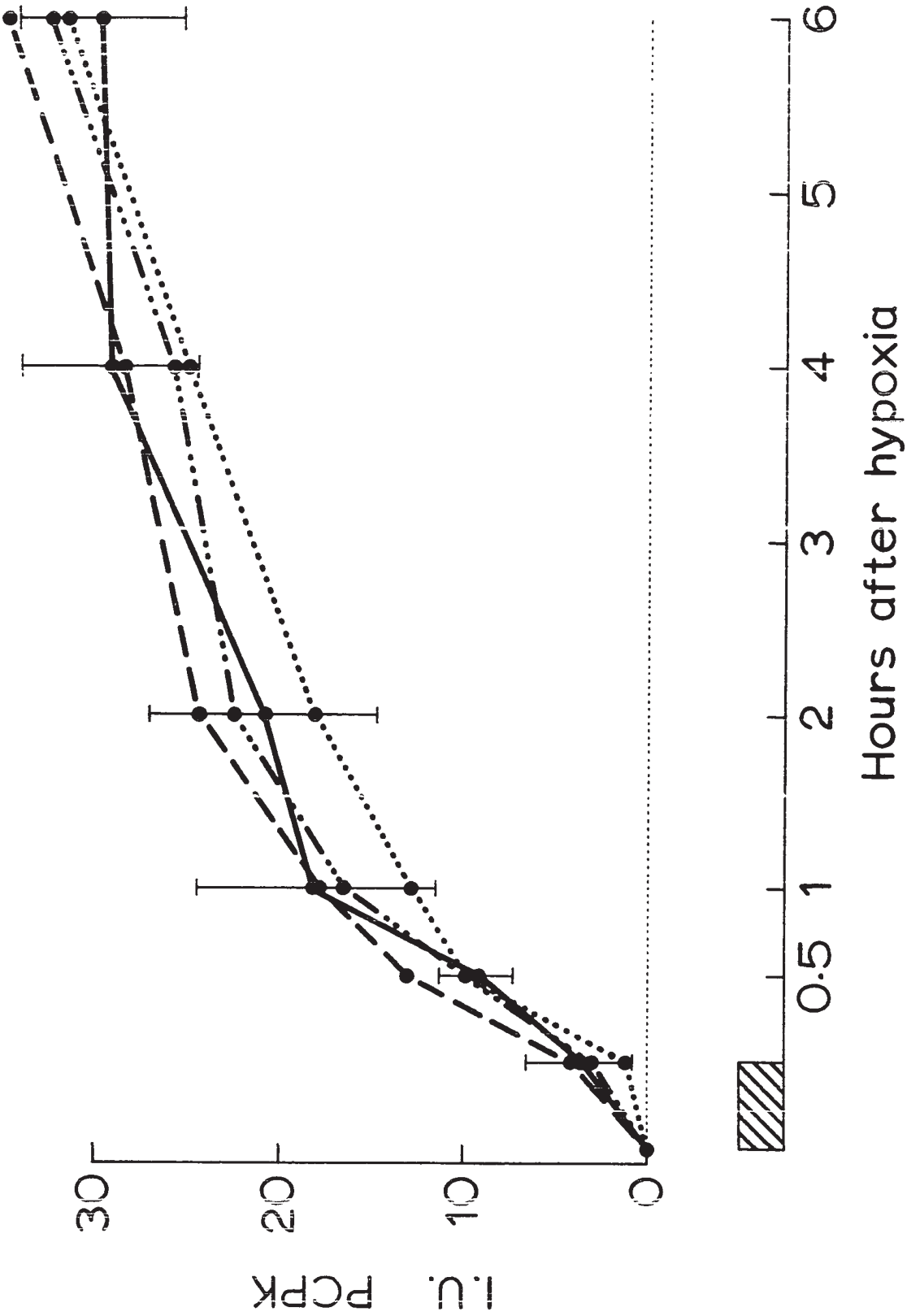
I.U. PCPK
30
20
10
0
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 11

PLDH activity of samples taken simultaneously from four different sampling sites in animals respired with five percent oxygen for 15 minutes.

Carotid Artery ———

Right Atrium .....

Femoral Vein — — —

Inferior Vena Cava -·-·-·-

Shaded area represents the duration of hypoxia.

Standard Error of the Mean is not included for the carotid artery, right atrium and inferior vena cava samples.

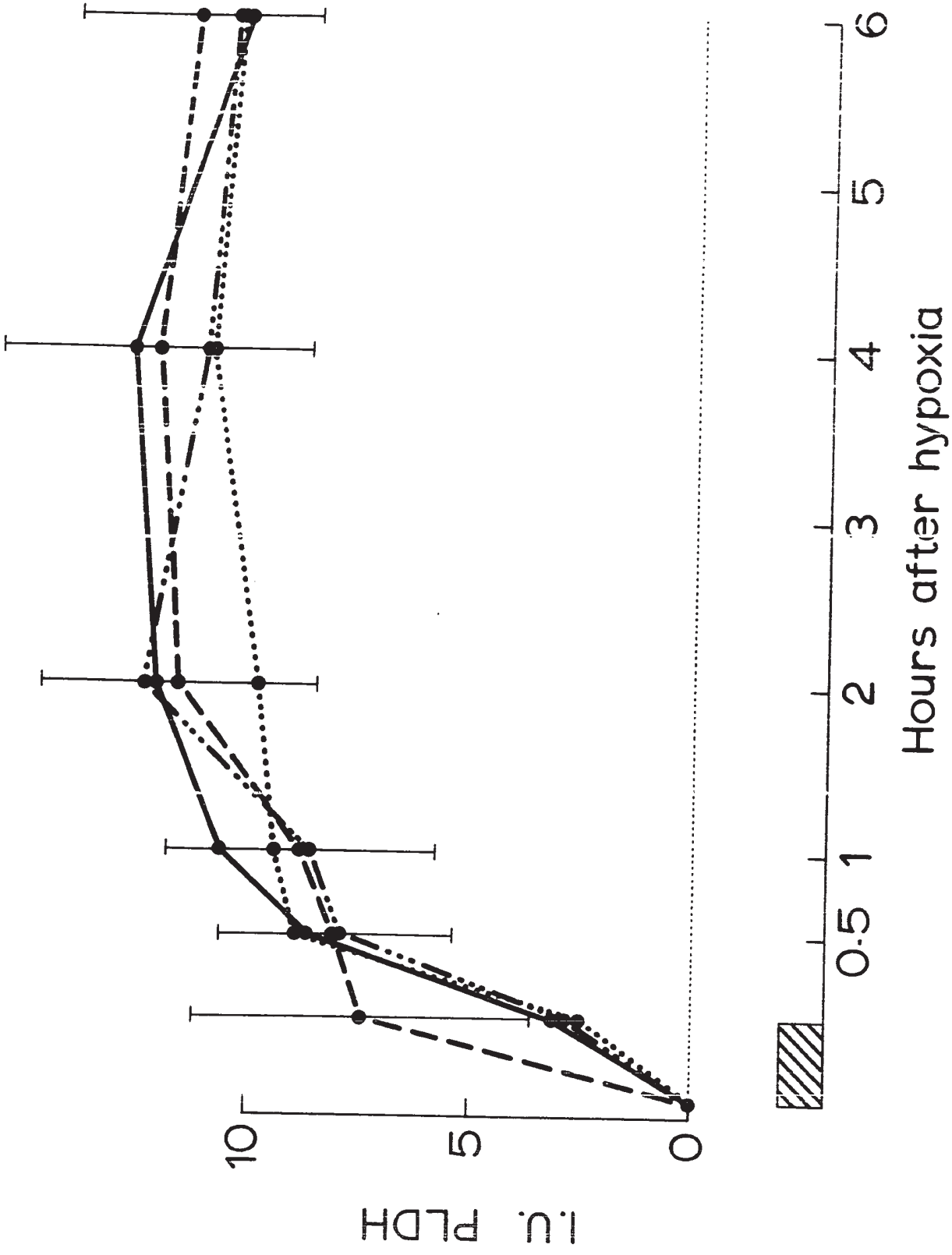
I.U. PLDH
10
5
0
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 12

PLDH isoenzyme ratio of samples taken simultaneously from four different sampling sites in animals respired with five percent oxygen for 15 minutes.

Carotid Artery _____

Right Atrium .....

Femoral Vein — — —

Inferior Vena Cava -·-·-·-

Shaded area represents the duration of hypoxia.

Standard error of the mean is not included for the right atrium and femoral vein samples.

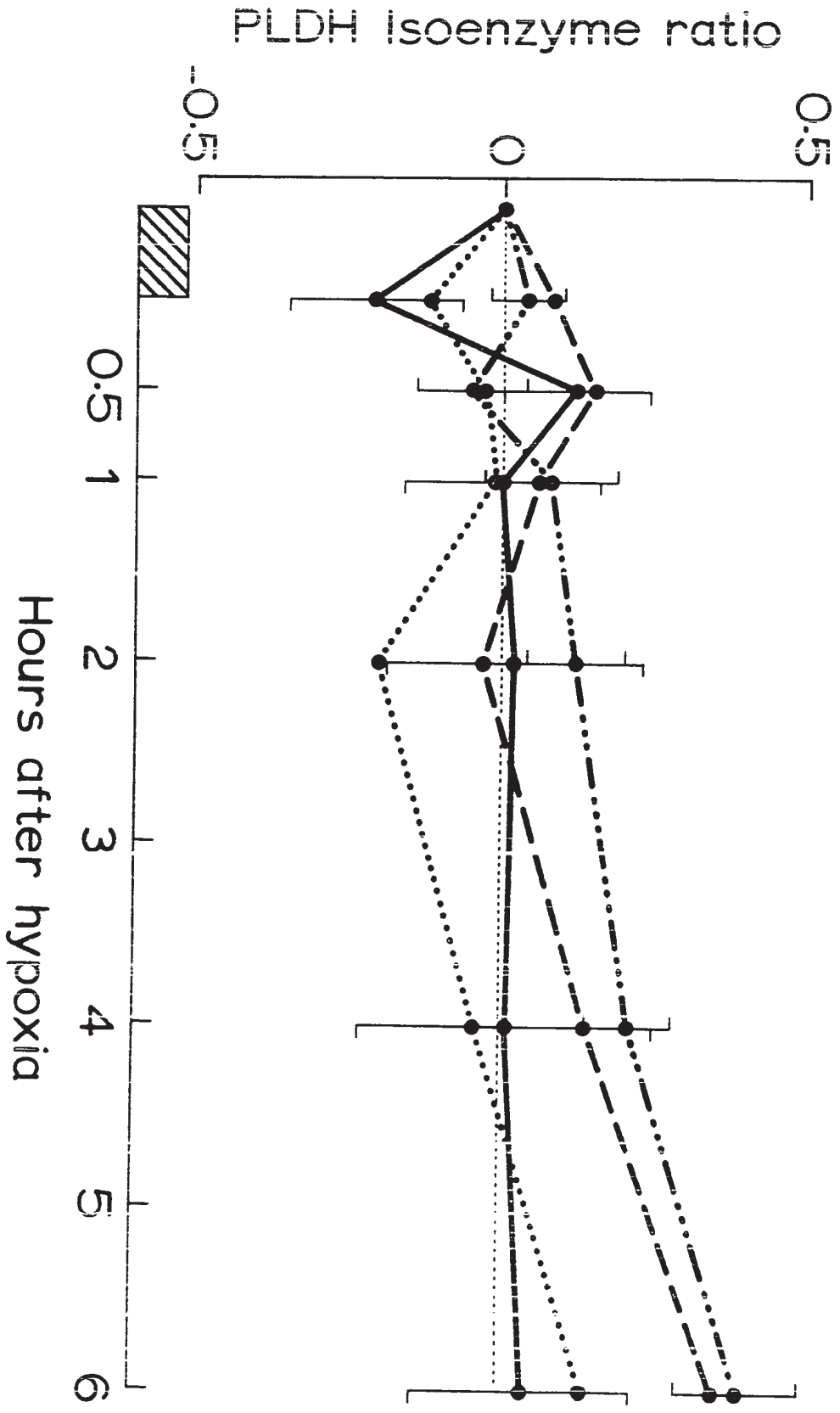
PLDH isoenzyme ratio
-0.5
0
0.5
Hours after hypoxia
0.5
1
2
3
4
5
6

TABLE 17

PGOT ACTIVITY OF BLOOD SAMPLES TAKEN SIMULTANEOUSLY FROM FOUR SAMPLING SITES IN ANIMALS RESPIRED WITH FIVE PERCENT OXYGEN FOR 15 MINUTES

Time	Carotid Artery 5% Oxygen	Right Atrium 5% Oxygen	Femoral Vein 5% Oxygen	Inferior Vena Cava 5% Oxygen
Before Hypoxia	0.0 ± 0.0*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
End of Hypoxia	2.7 ± 1.2	1.7 ± 1.0	2.6 ± 1.0	3.3 ± 0.6
1/2 Hour After Hypoxia	6.2 ± 1.3	6.8 ± 0.2	6.0 ± 0.8	5.9 ± 0.8
1 Hour	5.9 ± 0.7	6.1 ± 0.8	5.6 ± 0.3	7.0 ± 0.7
2 Hours	6.7 ± 0.5	6.4 ± 0.8	6.5 ± 0.5	8.4 ± 0.9
4 Hours	7.1 ± 1.5	6.7 ± 1.5	5.3 ± 1.0	7.9 ± 1.7
6 Hours	5.0 ± 1.7	5.6 ± 1.2	4.4 ± 0.9	7.5 ± 1.6

n is equal to 5.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

TABLE 18

PCPK ACTIVITY OF BLOOD SAMPLES TAKEN SIMULTANEOUSLY FROM FOUR SAMPLING SITES IN ANIMALS RESPIRED WITH FIVE PERCENT OXYGEN FOR 15 MINUTES

Time	Carotid Argery 5% Oxygen	Right Atrium 5% Oxygen	Femoral Vein 5% Oxygen	Inferior Vena Cava 5% Oxygen
Before Hypoxia	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
End of Hypoxia	3.4 ± 2.9*	1.0 ± 1.0	4.0 ± 1.7	3.0 ± 1.0
1/2 Hour After Hypoxia	9.2 ± 1.8	9.8 ± 2.7	13.0 ± 3.5	9.8 ± 1.7
1 Hour	18.4 ± 6.6	12.8 ± 4.3	17.8 ± 5.1	16.6 ± 4.4
2 Hours	20.8 ± 6.3	18.0 ± 5.6	24.2 ± 5.0	22.4 ± 4.3
4 Hours	29.2 ± 4.4	25.4 ± 6.8	28.4 ± 4.9	25.8 ± 4.7
6 Hours	29.8 ± 4.6	31.6 ± 5.6	34.8 ± 6.3	32.4 ± 5.5

n is equal to 5.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

TABLE 19

PLDH ACTIVITY OF BLOOD SAMPLES TAKEN SIMULTANEOUSLY FROM FOUR SAMPLING SITES IN ANIMALS RESPIRED WITH FIVE PERCENT OXYGEN FOR 15 MINUTES

Time	Carotid Artery 5% Oxygen	Right Atrium 5% Oxygen	Femoral Vein 5% Oxygen	Inferior Vena Cava 5% Oxygen
Before Hypoxia	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
End of Hypoxia	3.1 ± 1.5*	2.5 ± 1.4	7.4 ± 3.8	2.8 ± 1.1
1/2 Hour After Hypoxia	8.6 ± 1.5	8.9 ± 2.0	8.0 ± 2.6	7.9 ± 1.2
1 Hour	10.6 ± 1.9	9.4 ± 1.4	8.8 ± 3.0	8.6 ± 2.0
2 Hours	12.1 ± 2.4	9.8 ± 2.1	11.6 ± 3.1	12.3 ± 2.2
4 Hours	12.7 ± 1.7	10.9 ± 1.9	12.1 ± 3.4	10.9 ± 2.2
6 Hours	10.2 ± 1.2	10.3 ± 1.1	11.3 ± 2.7	10.4 ± 1.6

n is equal to 5.
Enzyme activity is expressed as International Units per liter of plasma.
*Standard Error of the Mean.

TABLE 20

PLDH ISOENZYME RATIO OF BLOOD SAMPLES TAKEN SIMULTANEOUSLY FROM FOUR SAMPLING SITES IN ANIMALS RESPIRED WITH FIVE PERCENT OXYGEN FOR 15 MINUTES

Time	Carotid Artery 5% Oxygen	Right Atrium 5% Oxygen	Femoral Vein 5% Oxygen	Inferior Vena Cava 5% Oxygen
Before Hypoxia	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
End of Hypoxia	-0.21 ± 0.14*	-0.12 ± 0.09	0.08 ± 0.12	0.04 ± 0.06
1/2 Hour After Hypoxia	0.12 ± 0.12	-0.03 ± 0.07	0.15 ± 0.10	-0.05 ± 0.09
1 Hour	0.00 ± 0.16	-0.01 ± 0.11	0.06 ± 0.06	0.08 ± 0.11
2 Hours	0.02 ± 0.21	-0.20 ± 0.05	-0.03 ± 0.11	0.12 ± 0.08
4 Hours	0.01 ± 0.24	-0.04 ± 0.09	0.14 ± 0.13	0.21 ± 0.07
6 Hours	0.04 ± 0.18	0.14 ± 0.11	0.35 ± 0.14	0.39 ± 0.10

n is equal to 5.
* Standard Error of the Mean.

FIGURE 13

PGOT activity of samples taken simultaneously from the carotid artery and right atrium in control animals.

Carotid Artery _____

Right Atrium .....

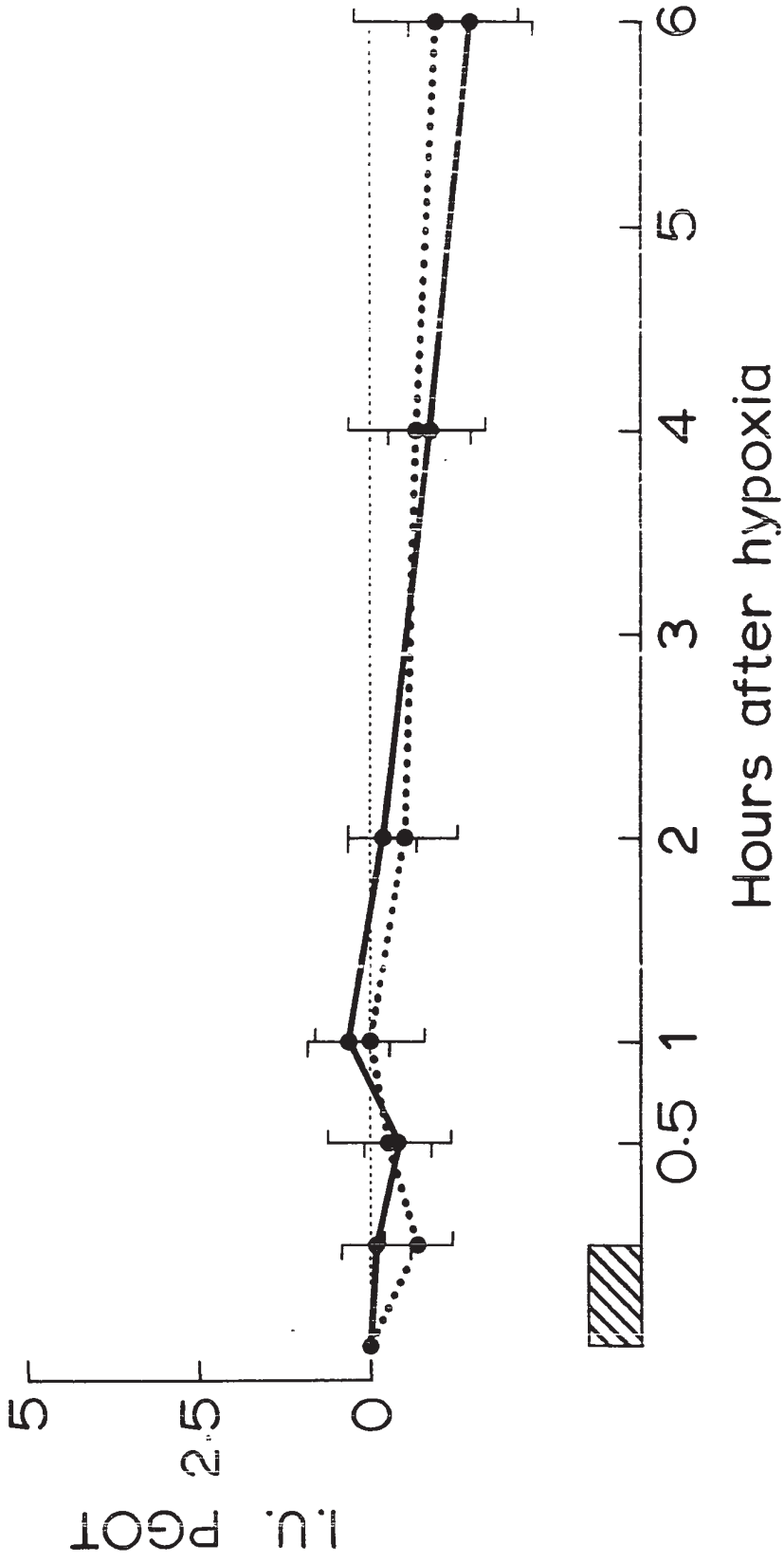
I.U. PGOT
5
2.5
0
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 14

PCPK activity of samples taken simultaneously from the carotid artery and right atrium in control animals.

Carotid Artery _____

Right Atrium .....

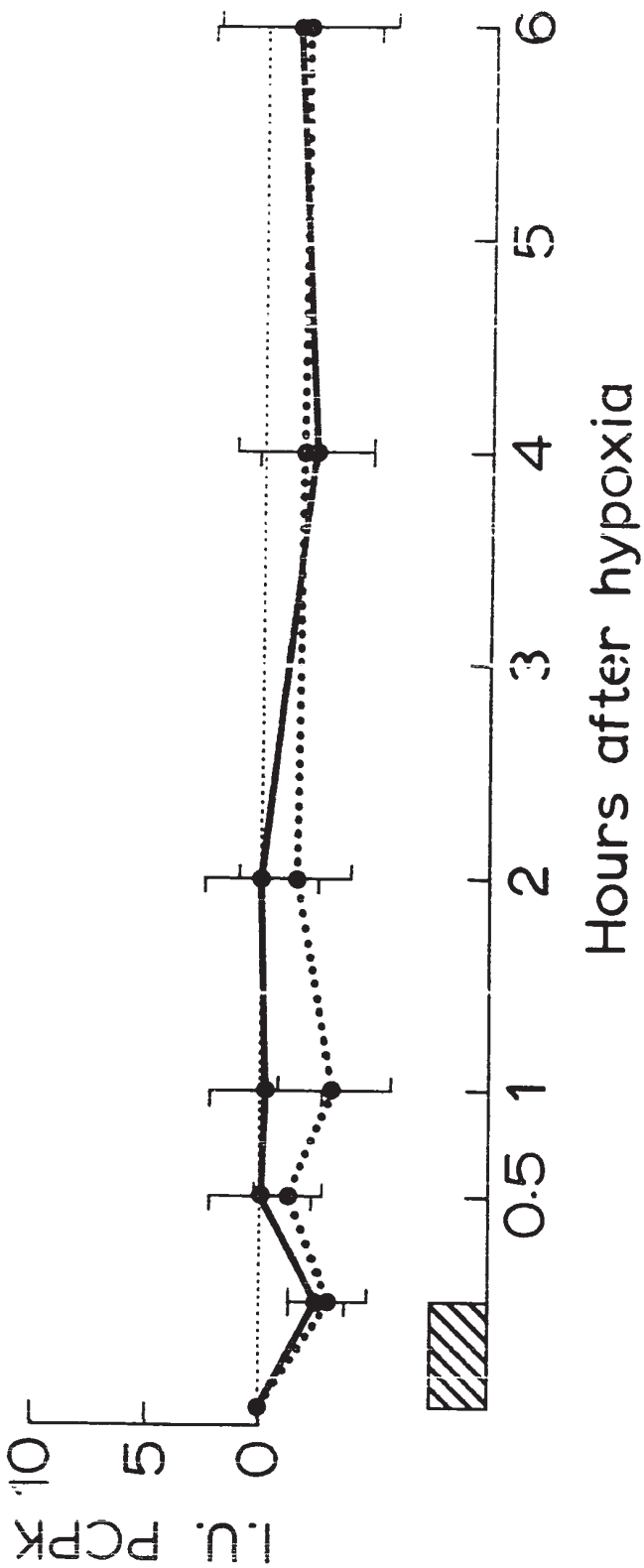
I.U. PCPK
10
5
0
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 15

PLDH activity of samples taken simultaneously from the carotid artery and right atrium in control animals.

Carotid Artery ———

Right Atrium .....

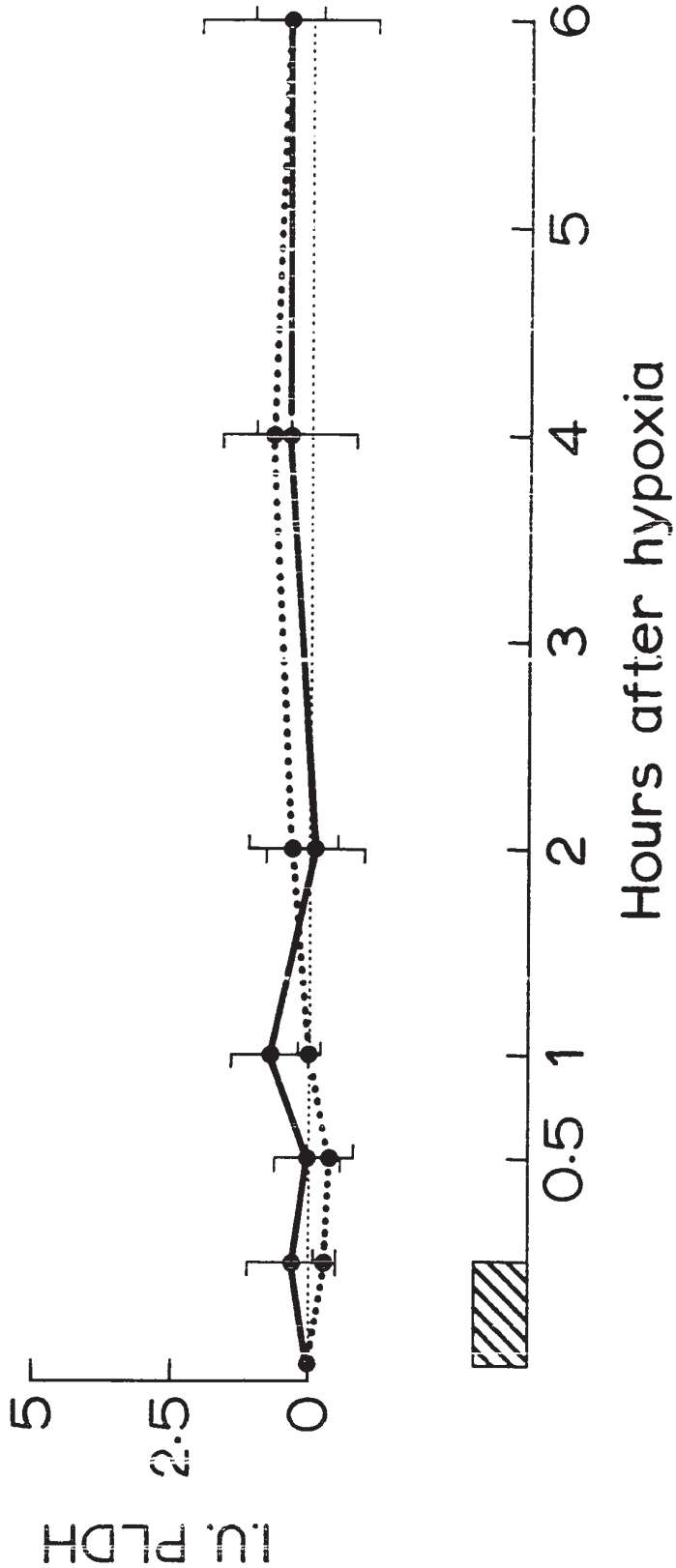
I.U. PLDH
5
2.5
0
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 16

PLDH isoenzyme ratio of samples taken simultaneously from the carotid artery and right atrium in control animals.

Carotid Artery _____

Right Atrium .....

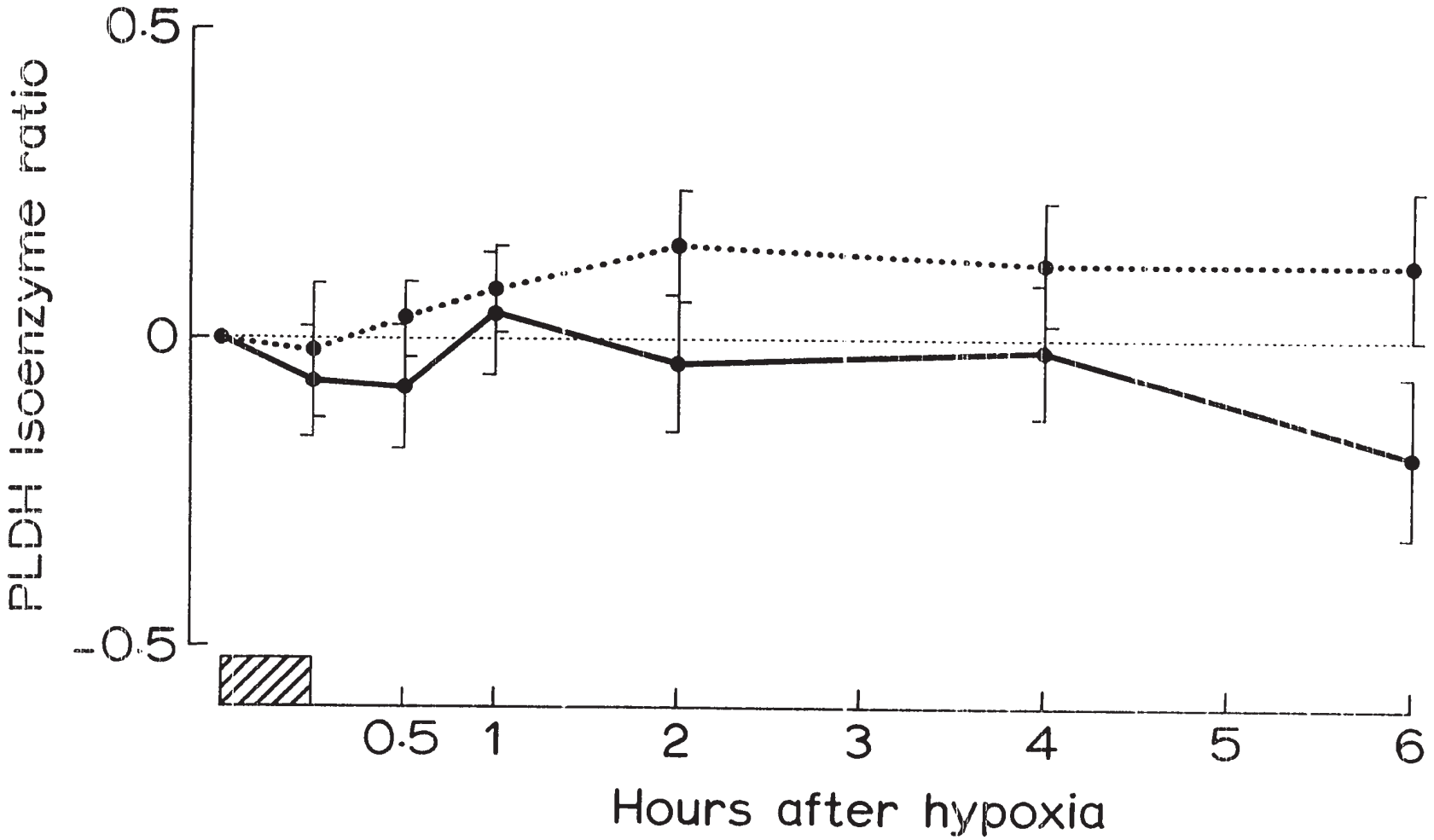
0.5
0
-0.5
PLDH isoenzyme ratio
0.5
1
2
3
4
5
6
Hours after hypoxia

TABLE 21

PGOT AND PCPK ACTIVITY OF BLOOD SAMPLES TAKEN SIMULTANEOUSLY FROM THE CAROTID ARTERY AND RIGHT ATRIUM IN CONTROL ANIMALS

Time	PGOT		PCPK	
	Carotid Artery	Right Atrium	Carotid Artery	Right Atrium
Before Hypoxia	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
End of Hypoxia	-0.1 ± 0.5*	-0.7 ± 0.5	-2.5 ± 1.1	-3.0 ± 1.7
1/2 Hour after Hypoxia	-0.4 ± 0.5	-0.3 ± 0.9	0.0 ± 2.2	-1.3 ± 1.5
1 Hour	0.3 ± 0.6	0.0 ± 0.8	-0.3 ± 2.5	-3.2 ± 2.4
2 Hours	-0.2 ± 0.5	-0.5 ± 0.8	0.0 ± 2.4	-1.5 ± 2.6
4 Hours	-0.9 ± 0.6	-0.7 ± 1.0	-2.2 ± 2.4	-1.8 ± 3.0
6 Hours	-1.5 ± 0.9	-1.0 ± 1.2	-1.5 ± 3.6	-1.7 ± 4.1

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
*Standard Error of the Mean.

TABLE 22

PLDH AND PLDH ISOENZYME RATIO OF BLOOD SAMPLES TAKEN SIMULTANEOUSLY FROM THE CAROTID ARTERY AND RIGHT ATRIUM IN CONTROL ANIMALS

Time	PLDH		PLDH ISOENZYME RATIO	
	Carotid Artery	Right Atrium	Carotid Artery	Right Atrium
Before Hypoxia	0.0 ± 0.0	0.0 ± 0.0	0.00 ± 0.00	0.00 ± 0.00
End of Hypoxia	0.3 ± 0.8*	-0.3 ± 0.2	-0.07 ± 0.09	-0.02 ± 0.11
1/2 Hour after Hypoxia	0.0 ± 0.6	-0.4 ± 0.4	-0.08 ± 0.10	0.03 ± 0.06
1 Hour	0.7 ± 0.7	0.0 ± 0.2	0.04 ± 0.10	0.08 ± 0.07
2 Hours	-0.1 ± 0.9	0.3 ± 0.8	-0.04 ± 0.11	0.15 ± 0.09
4 Hours	0.4 ± 1.2	0.7 ± 0.3	-0.02 ± 0.11	0.12 ± 0.10
6 Hours	0.4 ± 1.6	0.4 ± 0.6	-0.19 ± 0.13	0.12 ± 0.12

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

FIGURE 17

PGOT activity of samples taken simultaneously from the carotid artery and right atrium in animals respired with 10 percent oxygen for 30 minutes.

Carotid Artery -·-·-·-·

Right Atrium .....

Shaded area represents the duration of hypoxia.

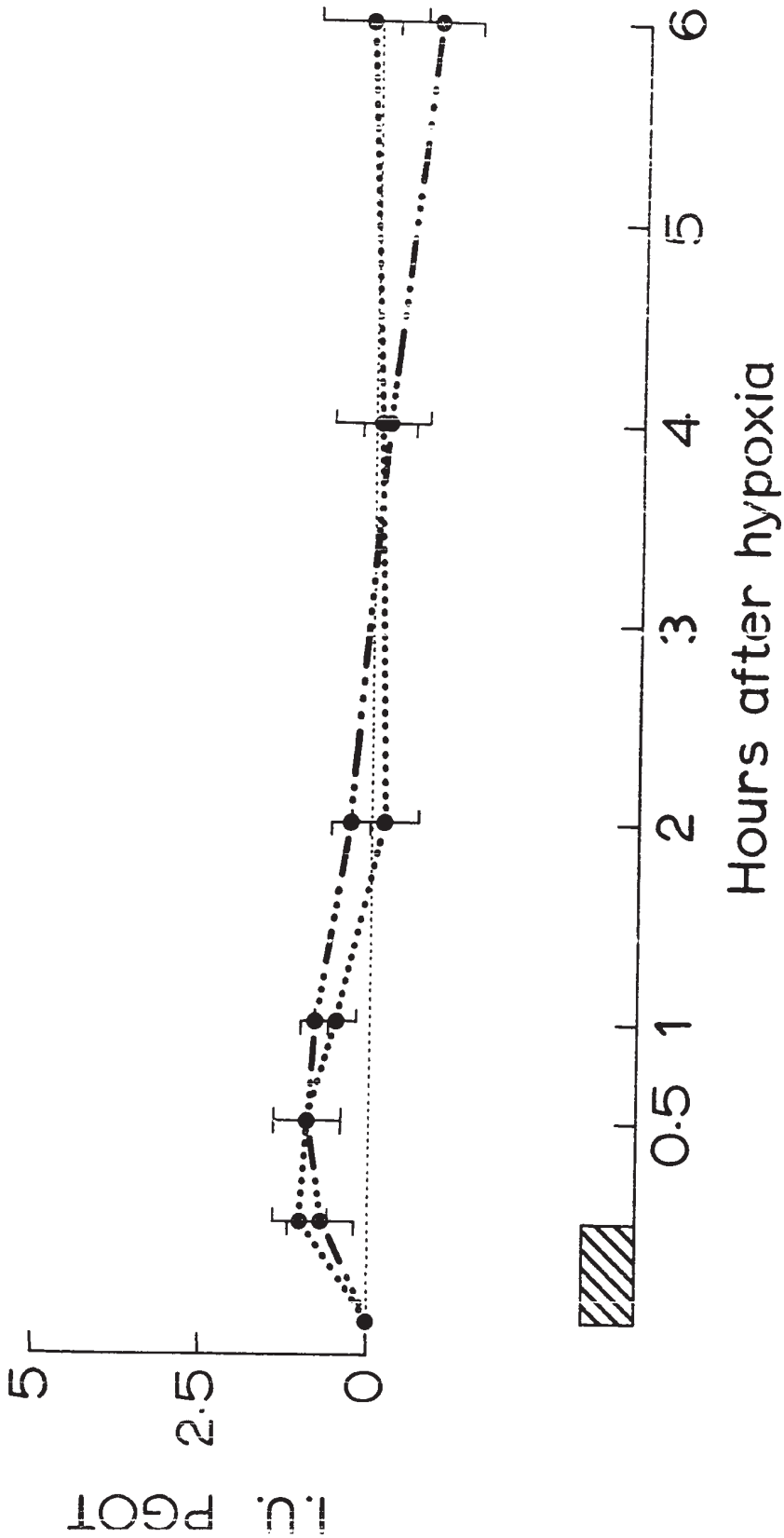
i.u. PGOT
5
2.5
0
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 18

PCPK activity of samples taken simultaneously from the carotid artery and right atrium in animals respired with 10 Percent oxygen for 30 minutes.

Carotid Artery -.....

Right Atrium .....

Shaded area represents the duration of hypoxia.

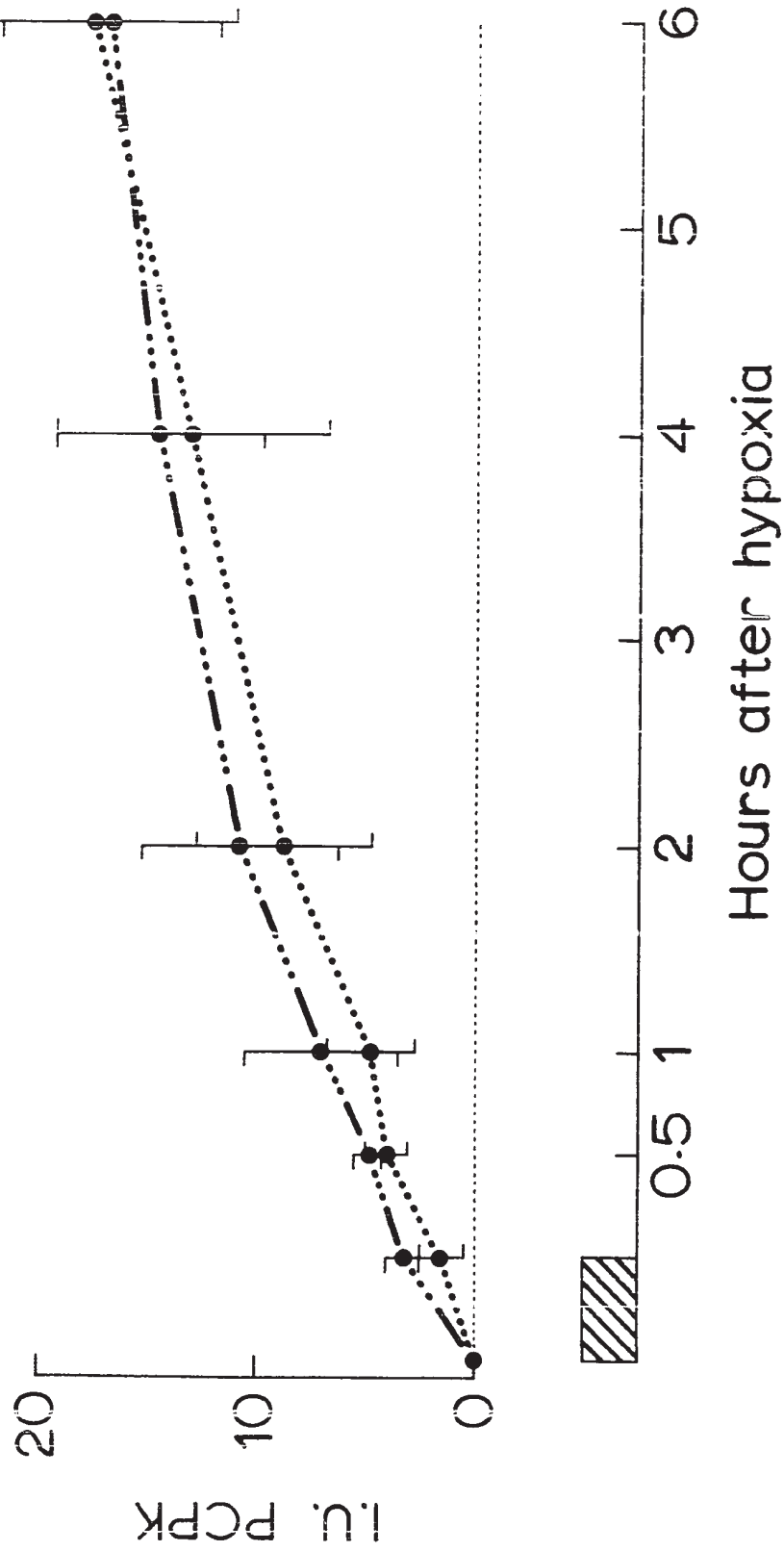
20
10
0
I.U. PCPK
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 19

PLDH activity of samples taken simultaneously from the carotid artery and right atrium in animals respired with 10 percent oxygen for 30 minutes.

Carotid Artery -.....

Right Atrium .....

Shaded area represents the duration of hypoxia.

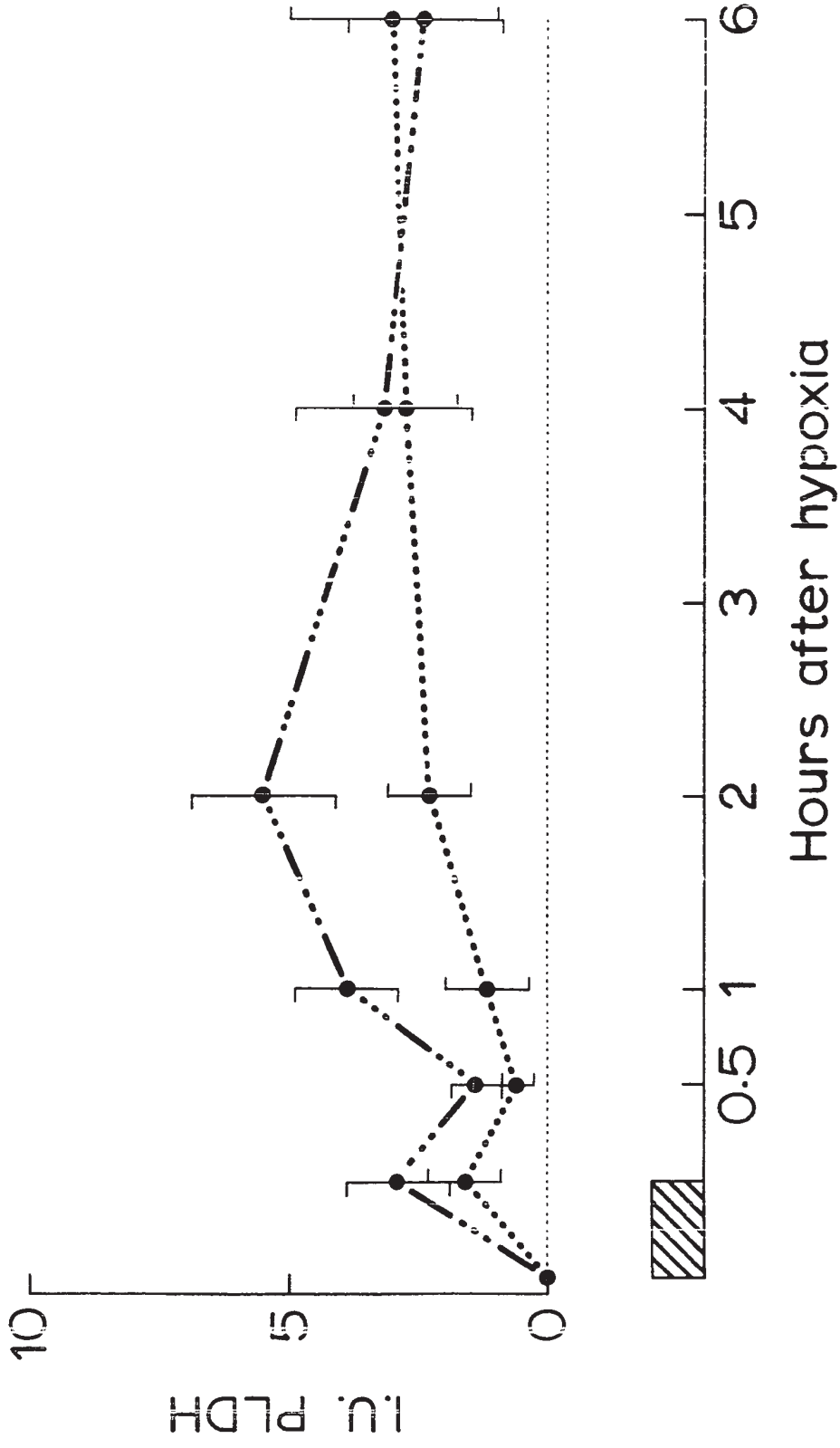
I.U. PLDH
10
5
0
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 20

PLDH isoenzyme ratio of samples taken simultaneously from the carotid artery and right atrium in animals respired with 10 percent oxygen for 30 minutes.

Carotid Artery -·-·-·-·-

Right Atrium . . . . . .

Shaded area represents the duration of hypoxia.

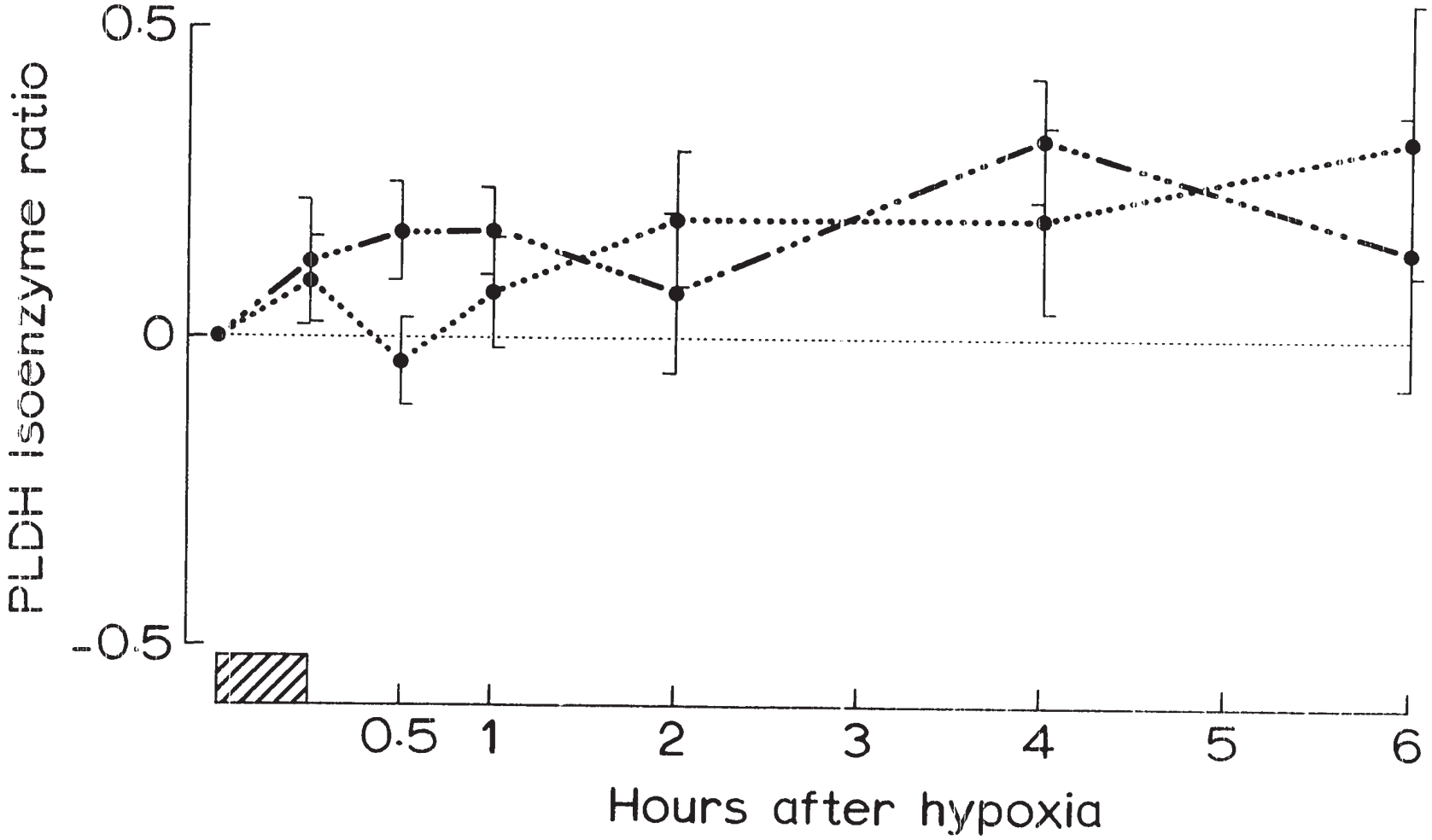
0·5
0
-0·5
PLDH isoenzyme ratio
0·5
1
2
3
4
5
6
Hours after hypoxia

TABLE 23

PGOT AND PCPK ACTIVITY OF BLOOD SAMPLES TAKEN SIMULTANEOUSLY FROM THE CAROTID ARTERY AND RIGHT ATRIUM IN ANIMALS RESPIRED WITH 10 PERCENT OXYGEN FOR 30 MINUTES

Time	PGOT		PCPK	
	Carotid Artery	Right Atrium	Carotid Artery	Right Atrium
Before Hypoxia	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
End of Hypoxia	0.7 ± 0.5*	1.0 ± 0.4	3.2 ± 0.6	1.7 ± 0.9
1/2 Hour After Hypoxia	0.9 ± 0.5	0.9 ± 0.5	4.7 ± 0.6	4.0 ± 0.9
1 Hour	0.8 ± 0.2	0.5 ± 0.3	7.0 ± 3.5	4.8 ± 1.9
2 Hours	0.3 ± 0.3	-0.2 ± 0.5	10.8 ± 4.5	8.8 ± 4.0
4 Hours	-0.2 ± 0.4	-0.1 ± 0.7	14.5 ± 4.8	13.0 ± 6.3
6 Hours	-0.9 ± 0.6	0.1 ± 0.8	16.7 ± 5.0	17.5 ± 6.6

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

TABLE 24

PLDH ACTIVITY AND PLDH ISOENZYME RATIO OF BLOOD SAMPLES TAKEN SIMULTANEOUSLY FROM THE CAROTID ARTERY AND RIGHT ATRIUM IN ANIMALS RESPIRED WITH 10 PERCENT OXYGEN FOR 30 MINUTES

Time	PLDH		PLDH ISOENZYME RATIO	
	Carotid Artery	Right Atrium	Carotid Artery	Right Atrium
Before Hypoxia	0.0 ± 0.0	0.0 ± 0.0	0.00 ± 0.00	0.00 ± 0.00
End of Hypoxia	2.9 ± 1.0*	1.6 ± 0.7**	0.12 ± 0.10	0.09 ± 0.07
1/2 Hour After Hypoxia	1.4 ± 0.5	0.6 ± 0.3	0.17 ± 0.08	-0.04 ± 0.07
1 Hour	3.9 ± 1.0	1.2 ± 0.8	0.17 ± 0.07	0.07 ± 0.09
2 Hours	5.5 ± 1.4	2.3 ± 0.8	0.07 ± 0.13	0.19 ± 0.11
4 Hours	3.2 ± 1.7	2.8 ± 1.0	0.32 ± 0.10	0.19 ± 0.15
6 Hours	2.4 ± 1.5	3.0 ± 2.0	0.14 ± 0.22	0.32 ± 0.22

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.
** P is less than 0.05.

(D) Prednisolone and Nitroglycerin

The animals receiving both prednisolone and nitroglycerin and ventilated with room air for 30 minutes (drug controls) showed no changes in plasma enzyme activity (Figures 21 to 24; Tables 25 and 26).

Prednisolone did not alter the PCPK, PLDH or PLDH isoenzyme ratio response to 7.5 percent oxygen (Figures 22 to 24; Tables 25 and 26). PGOT did not increase when compared with the drug control group (Figure 21; Table 25).

Nitroglycerin did not alter the PCPK response to 7.5 percent oxygen (Figure 22; Table 25). PGOT and PLDH did not increase when compared with the drug control group (Figures 21 and 23; Tables 25 and 26). The PLDH isoenzyme ratio increased in the animals given nitroglycerin while being ventilated with 7.5 percent oxygen (Figure 24; Table 26). This indicates that there was a proportionately greater release of LDH from skeletal muscle and liver tissue than there was from heart tissue.

Two of the six animals given nitroglycerin while being ventilated with 7.5 percent oxygen showed ischemic ECG changes, while four of the six animals pre-treated with prednisolone had ischemic ECG changes during the hypoxia.

The arterial oxygen tension at the end of hypoxia was higher in the animals receiving either prednisolone or nitroglycerin (17 mmHg)

than it was in the non-treated animals (11 mmHg). All three groups were ventilated with 7.5 percent oxygen for 30 minutes (Tables 5 and 27). There was no change in the arterial or venous carbon dioxide tensions or heart rate in the prednisolone or nitroglycerin-treated groups (Tables 28 and 29). The blood pressure response in the prednisolone-treated group was similar to the response of the non-treated group respired with 7.5 percent oxygen (Table 29). The nitroglycerin-treated group showed a smaller increase in peak blood pressure during the hypoxia (Table 29).

FIGURE 21

The effect of prednisolone or nitroglycerin on the PGOT response to hypoxia.

Drug Control ———

7.5 Percent Oxygen + Prednisolone — — —

7.5 Percent Oxygen + Nitroglycerin -·-·-·-

7.5 Percent Oxygen .....

Shaded area represents the duration of hypoxia.

Standard Error of the Mean is not included for the 7.5 percent oxygen + nitroglycerin and 7.5 percent oxygen groups.

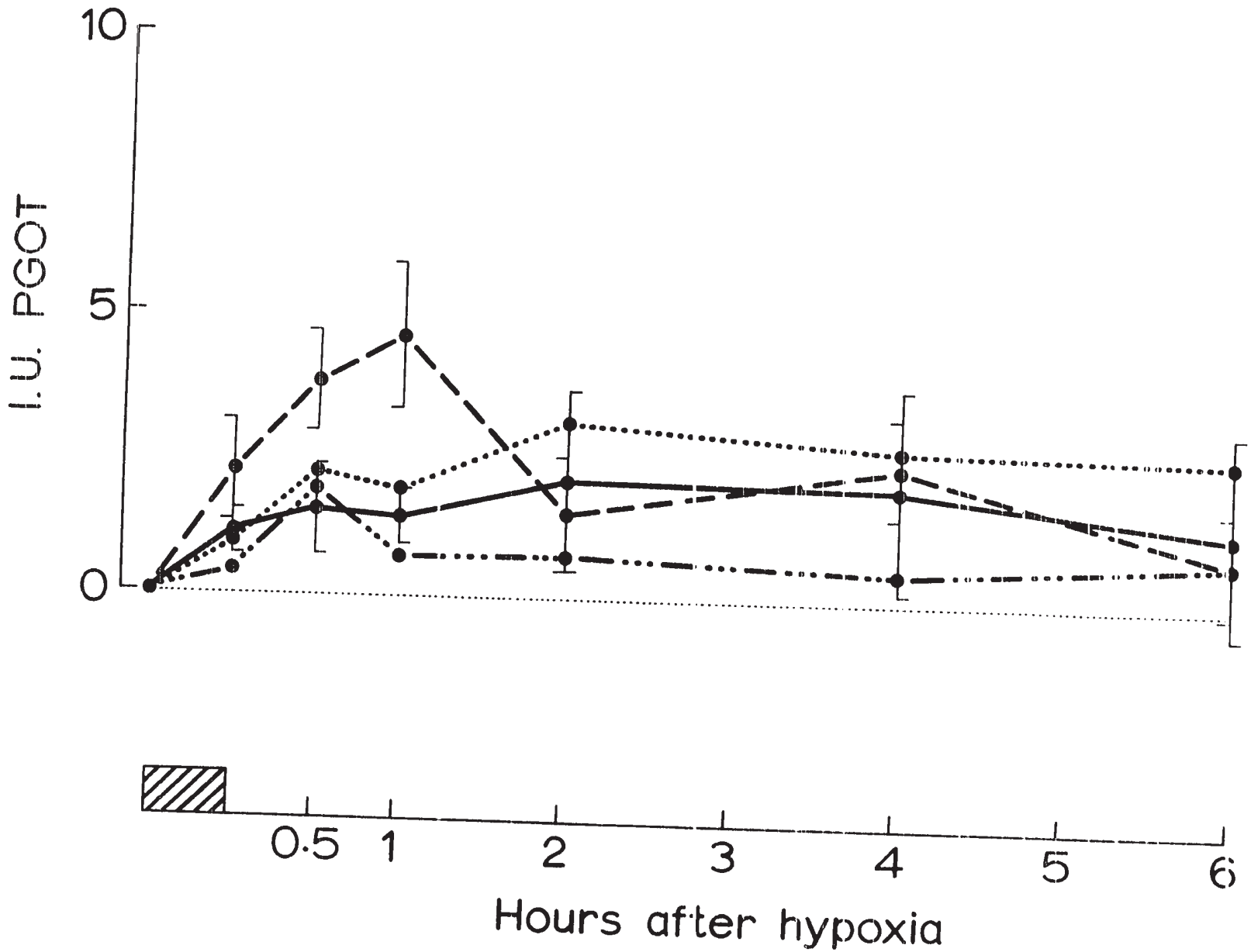
10
5
0
I.U. PGOT
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 22

The effect of prednisolone or nitroglycerin on the PCPK response to hypoxia.

Drug Control ———

7.5 Percent Oxygen + Prednisolone — — —

7.5 Percent Oxygen + Nitroglycerin -.-.-.-.-

7.5 Percent Oxygen .....

Shaded area represents the duration of hypoxia.

Standard Error of the Mean is not included for the 7.5 percent oxygen group.

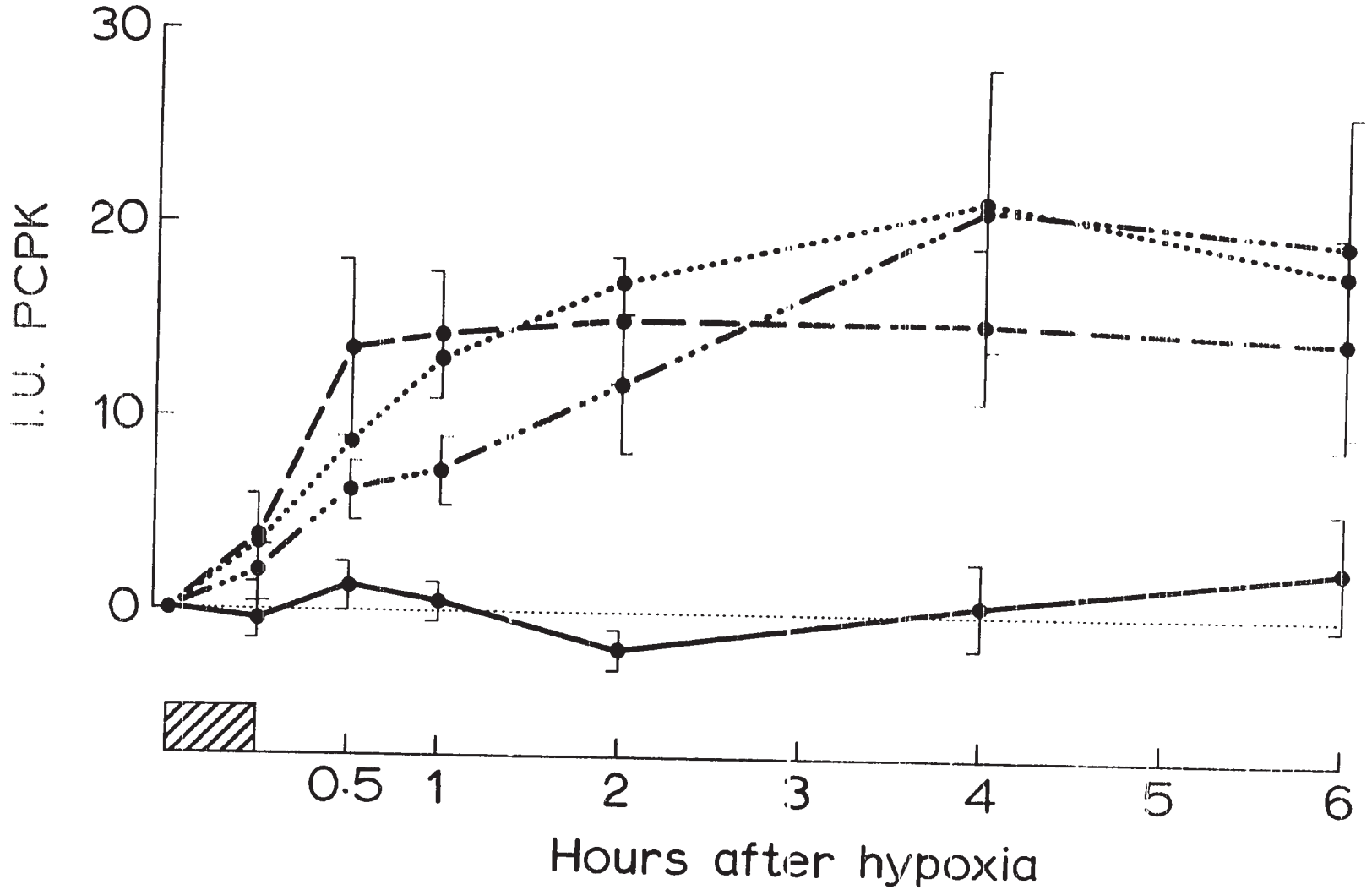
30
20
10
0
I.U. PCPK
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 23

The effect of prednisolone or nitroglycerin on the PLDH response to hypoxia.

Drug Control ———

7.5 Percent Oxygen + Prednisolone — — —

7.5 Percent Oxygen + Nitroglycerin -.-.-.-

7.5 Percent Oxygen .....

Shaded area represents the duration of hypoxia.

Standard Error of the Mean is not included for the 7.5 Percent Oxygen group.

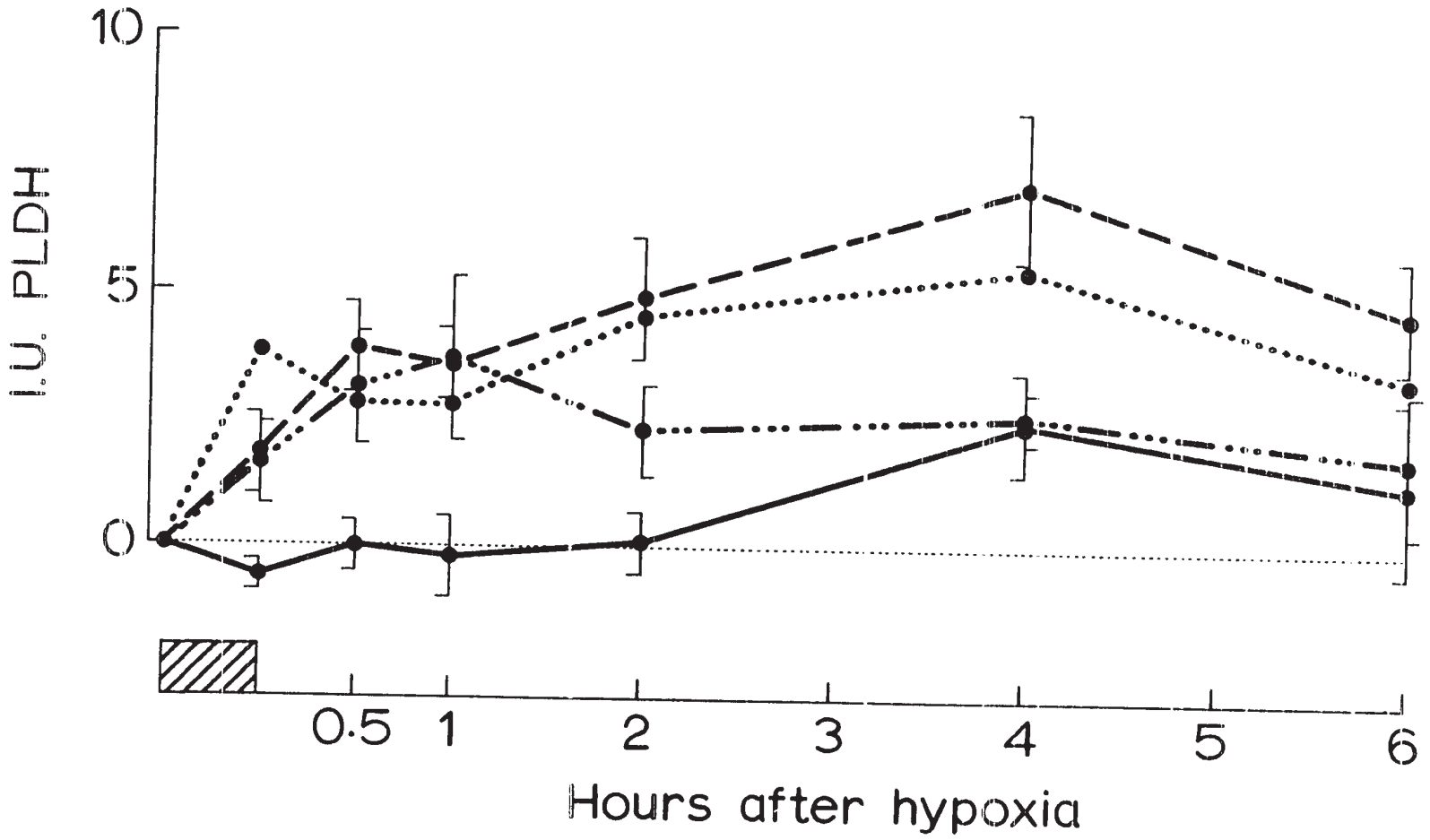
10
5
0
I.U. PLDH
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 24

The effect of prednisolone or nitroglycerin on the PLDH isoenzyme ratio response to hypoxia.

Drug Control ———

7.5 Percent Oxygen + Prednisolone — — —

7.5 Percent Oxygen + Nitroglycerin -...-...

Shaded area represents the duration of hypoxia.

Standard Error of the Mean is not included for the 7.5 Percent oxygen + prednisolone group.

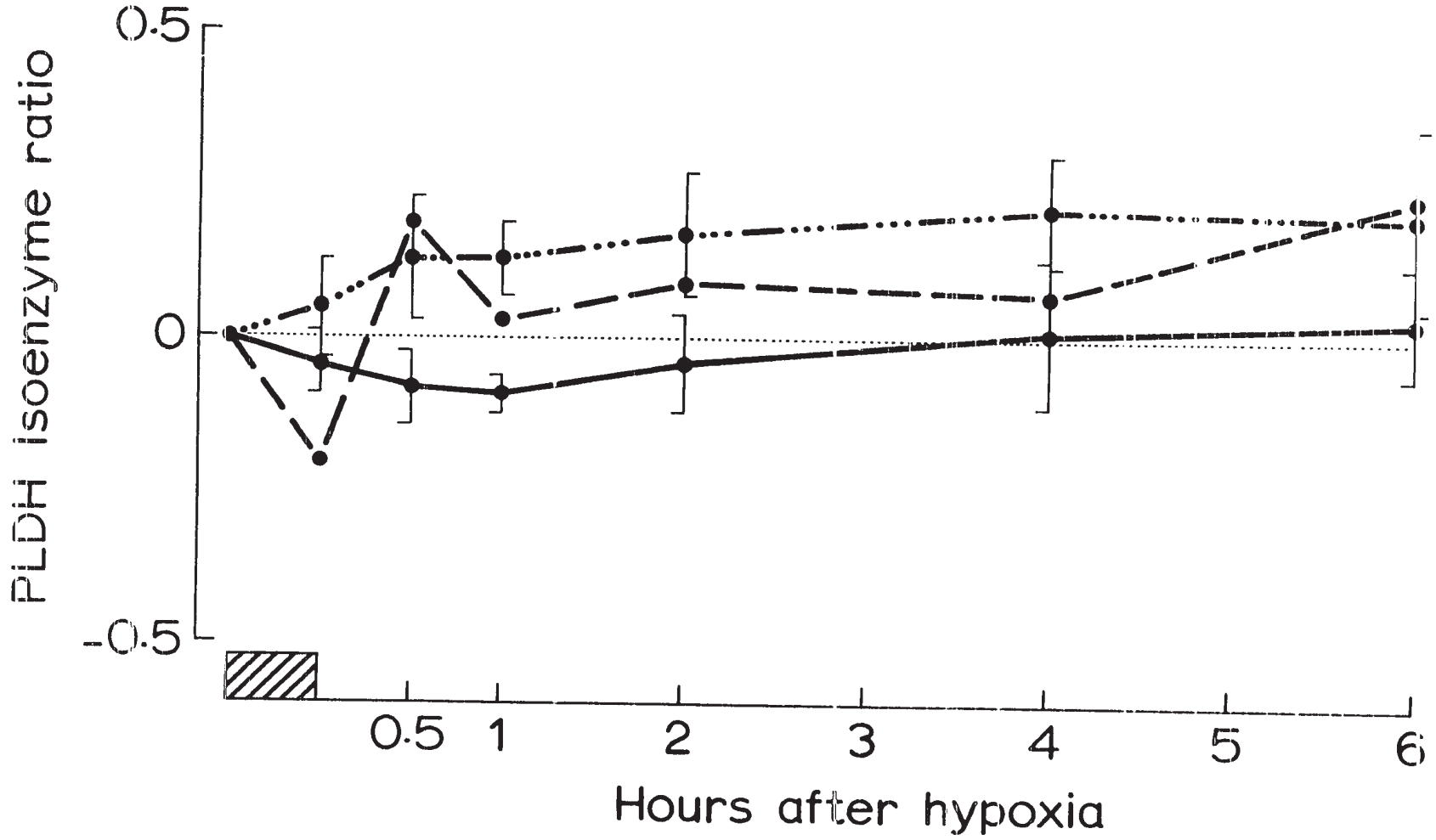
0.5
0
-0.5
PLDH isoenzyme ratio
0.5
1
2
3
4
5
6
Hours after hypoxia

TABLE 25

THE EFFECT OF PREDNISOLONE OR NITROGLYCERIN ON THE PGOT AND PCPK RESPONSE TO HYPOXIA

Time	PGOT			PCPK		
	21% Oxygen Prednisolone & Nitro-glycerin (Drug Control)	7.5% Oxygen Prednisolone	7.5% Oxygen Nitroglycerin	21% Oxygen Prednisolone & Nitro-glycerin (Drug Control)	7.5% Oxygen Prednisolone	7.5% Oxygen Nitroglycerin
Before Hypoxia	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
End of Hypoxia	1.1 ± 0.4*	2.2 ± 0.9	0.4 ± 0.7	-0.5 ± 0.7	3.5 ± 2.1**	2.0 ± 1.5**
1/2 Hour After Hypoxia	1.5 ± 0.8	3.8 ± 0.9	1.9 ± 0.6	1.3 ± 1.2	13.5 ± 4.5	6.3 ± 1.6
1 Hour	1.4 ± 0.5	4.6 ± 1.3	0.7 ± 0.9	0.5 ± 0.9	14.2 ± 3.1	7.3 ± 1.8
2 Hours	2.1 ± 1.6	1.5 ± 1.0	0.7 ± 0.9	-0.8 ± 1.0	15.0 ± 3.3	11.7 ± 3.4
4 Hours	2.0 ± 1.8	2.4 ± 0.9	0.5 ± 0.7	0.2 ± 2.1	15.0 ± 4.0	21.0 ± 7.3
6 Hours	1.3 ± 1.8	0.8 ± 0.9	0.8 ± 0.8	2.5 ± 2.9	14.2 ± 5.2	19.3 ± 8.2

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.
** P is less than 0.05.

TABLE 26

THE EFFECT OF PREDNISOLONE OR NITROGLYCERIN ON THE PLDH AND PLDH ISOENZYME RATIO RESPONSE TO HYPOXIA

Time	PLDH			PLDH Isoenzyme Ratio		
	21% Oxygen Prednisolone & Nitro-glycerin (Drug Control)	7.5% Oxygen Prednisolone	7.5% Oxygen Nitroglycerin	21% Oxygen Prednisolone & Nitro-glycerin (Drug Control)	7.5% Oxygen Prednisolone	7.5% Oxygen Nitroglycerin
Before Hypoxia	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	00.0 ± 0.00	00.0 ± 0.00	00.0 ± 0.00
End of Hypoxia	-0.6 ± 0.2*	1.8 ± 0.8**	1.6 ± 0.8	-0.04 ± 0.05	-0.20 ± 0.08	0.05 ± 0.08**
1/2 Hour After Hypoxia	0.0 ± 0.5	3.9 ± 0.9	3.1 ± 1.1	-0.08 ± 0.06	0.19 ± 0.10	0.13 ± 0.13
1 Hour	-0.2 ± 0.8	3.6 ± 0.7	3.7 ± 1.6	-0.09 ± 0.03	0.03 ± 0.06	0.13 ± 0.09
2 Hours	0.1 ± 0.6	4.9 ± 1.2	2.3 ± 0.9	-0.04 ± 0.08	0.09 ± 0.09	0.17 ± 0.10
4 Hours	2.5 ± 1.0	7.1 ± 1.4	2.6 ± 0.5	0.01 ± 0.12	0.07 ± 0.09	0.21 ± 0.03
6 Hours	1.3 ± 1.7	4.7 ± 1.1	1.8 ± 1.4	0.03 ± 0.09	0.23 ± 0.15	0.20 ± 0.10

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.
** P less than 0.05.

TABLE 27

THE EFFECT OF PREDNISOLONE OR NITROGLYCERIN ON THE ARTERIAL AND VENOUS OXYGEN TENSION RESPONSE TO HYPOXIA

Time	Arterial Oxygen			Venous Oxygen		
	21% Oxygen Prednisolone & Nitro-glycerin (Drug Control)	7.5% Oxygen Prednisolone	7.5% Oxygen Nitroglycerin	21% Oxygen Prednisolone & Nitro-glycerin (Drug Control)	7.5% Oxygen Prednisolone	7.5% Oxygen Nitroglycerin
Before Hypoxia	65 ± 1*	78 ± 5	72 ± 3	47 ± 1	39 ± 7	42 ± 2
End of Hypoxia	69 ± 2	17 ± 2	17 ± 2	41 ± 1	10 ± 2	10 ± 2
1/2 Hour After Hypoxia	70 ± 2	69 ± 3	70 ± 3	48 ± 1	46 ± 2	41 ± 3
1 Hour	70 ± 2	70 ± 3	75 ± 2	48 ± 2	47 ± 3	45 ± 2
2 Hours	69 ± 2	69 ± 1	73 ± 2	46 ± 2	44 ± 3	43 ± 1
4 Hours	69 ± 3	68 ± 2	67 ± 3	44 ± 2	43 ± 1	41 ± 2
6 Hours	66 ± 2	69 ± 2	69 ± 2	46 ± 1	43 ± 2	40 ± 1

n is equal to 6.
Oxygen tension is expressed as mmHg.
* Standard Error of the Mean.

TABLE 28

THE EFFECT OF PREDNISOLONE OR NITROGLYCERIN ON THE ARTERIAL AND VENOUS CARBON DIOXIDE TENSION RESPONSE TO HYPOXIA

Time	Arterial Carbon Dioxide			Venous Carbon Dioxide		
	21% Oxygen Prednisolone & Nitro-glycerin (Drug Control)	7.5% Oxygen Prednisolone	7.5% Oxygen Nitroglycerin	21% Oxygen Prednisolone & Nitro-glycerin (Drug Control)	7.5% Oxygen Prednisolone	7.5% Oxygen Nitroglycerin
Before Hypoxia	35 ± 1*	31 ± 1	33 ± 2	39 ± 1	35 ± 1	37 ± 2
End of Hypoxia	35 ± 1	30 ± 1	32 ± 2	40 ± 1	34 ± 1	37 ± 3
1/2 Hour After Hypoxia	36 ± 2	32 ± 1	34 ± 1	41 ± 2	35 ± 1	40 ± 2
1 Hour	35 ± 2	31 ± 1	34 ± 1	38 ± 2	35 ± 1	37 ± 2
2 Hours	35 ± 1	30 ± 1	37 ± 2	38 ± 2	34 ± 1	43 ± 3
4 Hours	30 ± 1	29 ± 1	37 ± 2	35 ± 1	32 ± 1	42 ± 2
6 Hours	29 ± 2	29 ± 2	36 ± 2	33 ± 2	34 ± 1	42 ± 3

n is equal to 6.
Carbon dioxide tension is expressed as mmHg.
* Standard Error of the Mean.

TABLE 29

THE EFFECT OF PREDNISOLONE OR NITROGLYCERIN ON THE ARTERIAL SYSTOLIC AND DIASTOLIC BLOOD PRESSURE AND HEART RATE RESPONSE TO HYPOXIA

	Systolic Blood Pressure			Diastolic Blood Pressure			Heart Rate		
Time	21% Oxygen Prednisolone & Nitroglycerin (Drug Control)	7.5% Oxygen Prednisolone	7.5% Oxygen Nitroglycerin	21% Oxygen Prednisolone & Nitroglycerin (Drug Control)	7.5% Oxygen Prednisolone	7.5% Oxygen Nitroglycerin	21% Oxygen Prednisolone & Nitroglycerin (Drug Control)	7.5% Oxygen Prednisolone	7.5% Oxygen Nitroglycerin
Before Hypoxia	131±5*	138±5	128±8	110±3	118±4	108±6	161±5	175±9	177±18
Peak Blood Pressure		198±6 (5 min.)	157±14 (7 min.)***		141±5 (5 min.)	117±8 (7 min.)***		145±9 (5 min.)	163±8 (7 min.)***
End of Hypoxia	112±6	113±11	103±19	99±4	86±8	82±16	159±8	154±14	167±19
1/2 Hour After Hypoxia	115±8	130±15	115±8	98±4	100±10	101±7	147±9	161±10	184±19
1 Hour	129±6	127±14	120±7	107±5	101±10	103±5	147±10	159±8	188±18
2 Hours	126±5	137±13	121±7	102±3	109±7	103±5	145±10	175±10	173±20
4 Hours	124±4	139±11	131±9	103±2	113±8	108±4	143±13	148±7	165±14
6 Hours	132±6	138±10	129±9	108±5	109±8	109±6	153±16	139±5	161±18

n is equal to 6.
Blood Pressure is expressed as mmHg.
Heart rate is expressed as beats per minute.

* Standard Error of the Mean.
*** Time after beginning of hypoxia when peak blood pressure was reached.

(E) Succinylcholine

The possibility that succinylcholine was modifying the plasma enzyme response to hypoxia was investigated by eliminating this drug in a group of animals ventilated with 10 percent oxygen. Succinylcholine had no effect on the plasma enzyme response to 10 percent oxygen (Figures 25 to 28; Tables 30 and 31). The degree of hypoxia, as judged by the blood oxygen tensions at the end of the hypoxia, was very similar for the two groups ventilated with 10 percent oxygen (Table 32). There was no change in the arterial or venous carbon dioxide tensions in the group not receiving succinylcholine (Table 33). The blood pressure and heart rate changes in this group were similar to those seen in the group given succinylcholine and ventilated with 10 percent oxygen (Table 34).

FIGURE 25

The effect of succinylcholine on the PGOT response to hypoxia.

Control ———

10 Percent oxygen with succinylcholine —·—·—

10 Percent oxygen without succinylcholine .....

Shaded area represents the duration of hypoxia.

Standard Error of the Mean is not included for the 10 Percent oxygen with succinylcholine group.

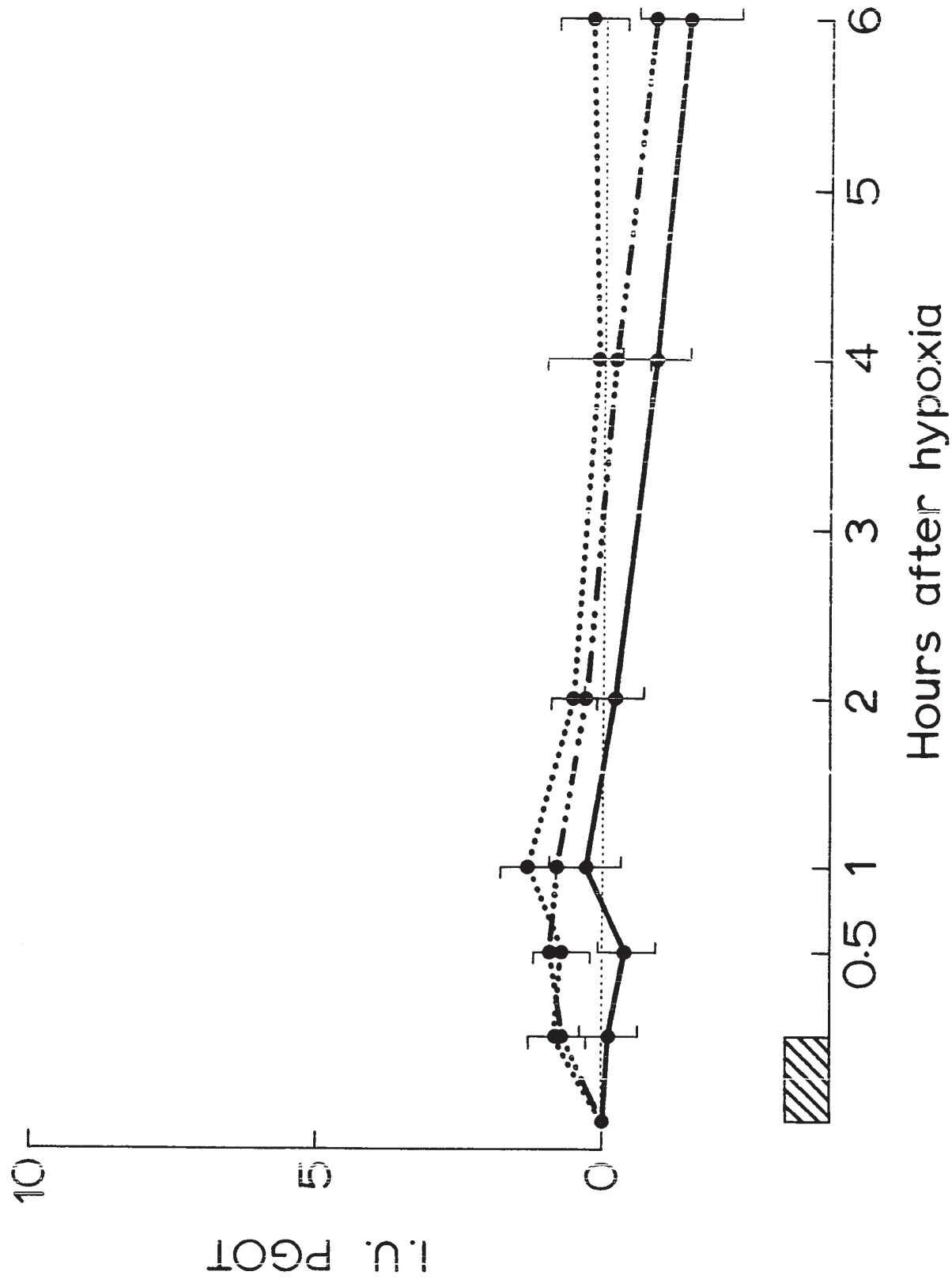
10
5
0
I.U. PGOT
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 26

The effect of succinylcholine on the PCPK response to hypoxia.

Control ———

10 Percent oxygen with succinylcholine -··· -··-

10 Percent oxygen without succinylcholine ·····

Shaded area represents the duration of hypoxia.

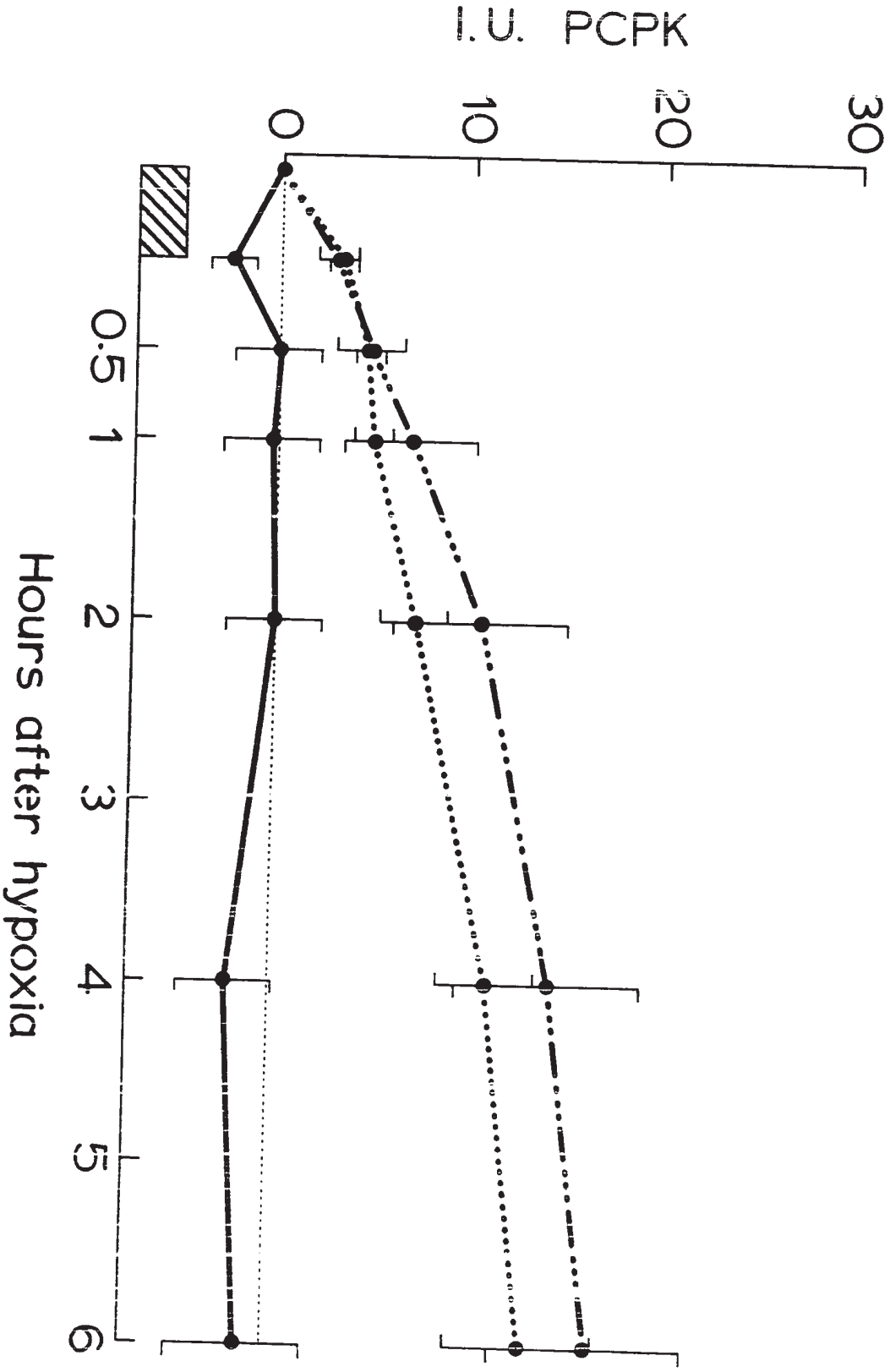
I.U. PCPK
0
10
20
30
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 27

The effect of succinylcholine on the PLDH response to hypoxia.

Control ———

10 Percent oxygen with succinylcholine -.... -....

10 Percent oxygen without succinylcholine ......

Shaded area represents the duration of hypoxia.

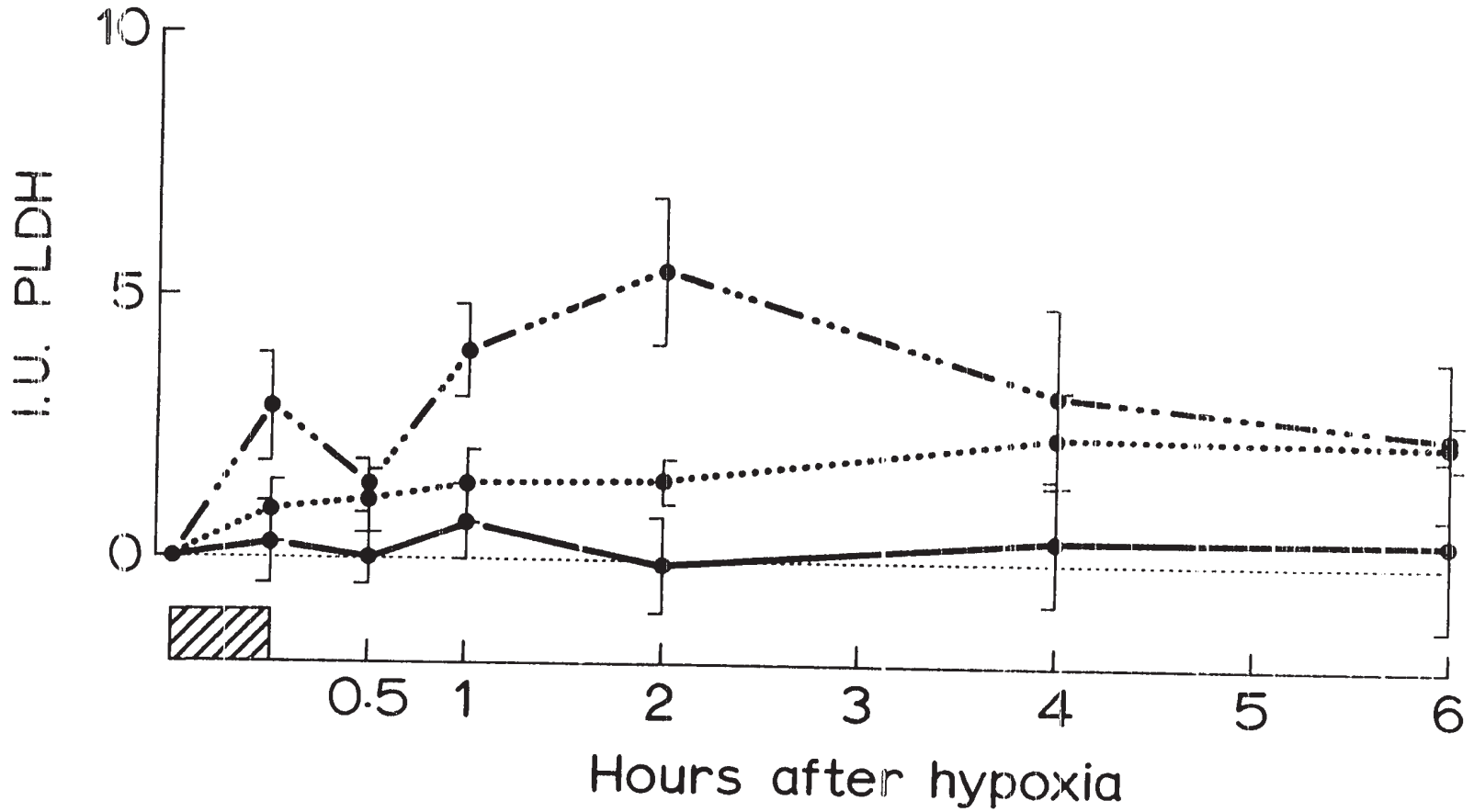
10
5
0
I.U. PLDH
0.5
1
2
3
4
5
6
Hours after hypoxia

FIGURE 28

The effect of succinylcholine on the PLDH isoenzyme ratio response to hypoxia.

Control ———

10 Percent oxygen with succinylcholine -.-.-.-

10 Percent oxygen without succinylcholine .....

Shaded area represents the duration of hypoxia.

Standard Error of the Mean is not included for the control group.

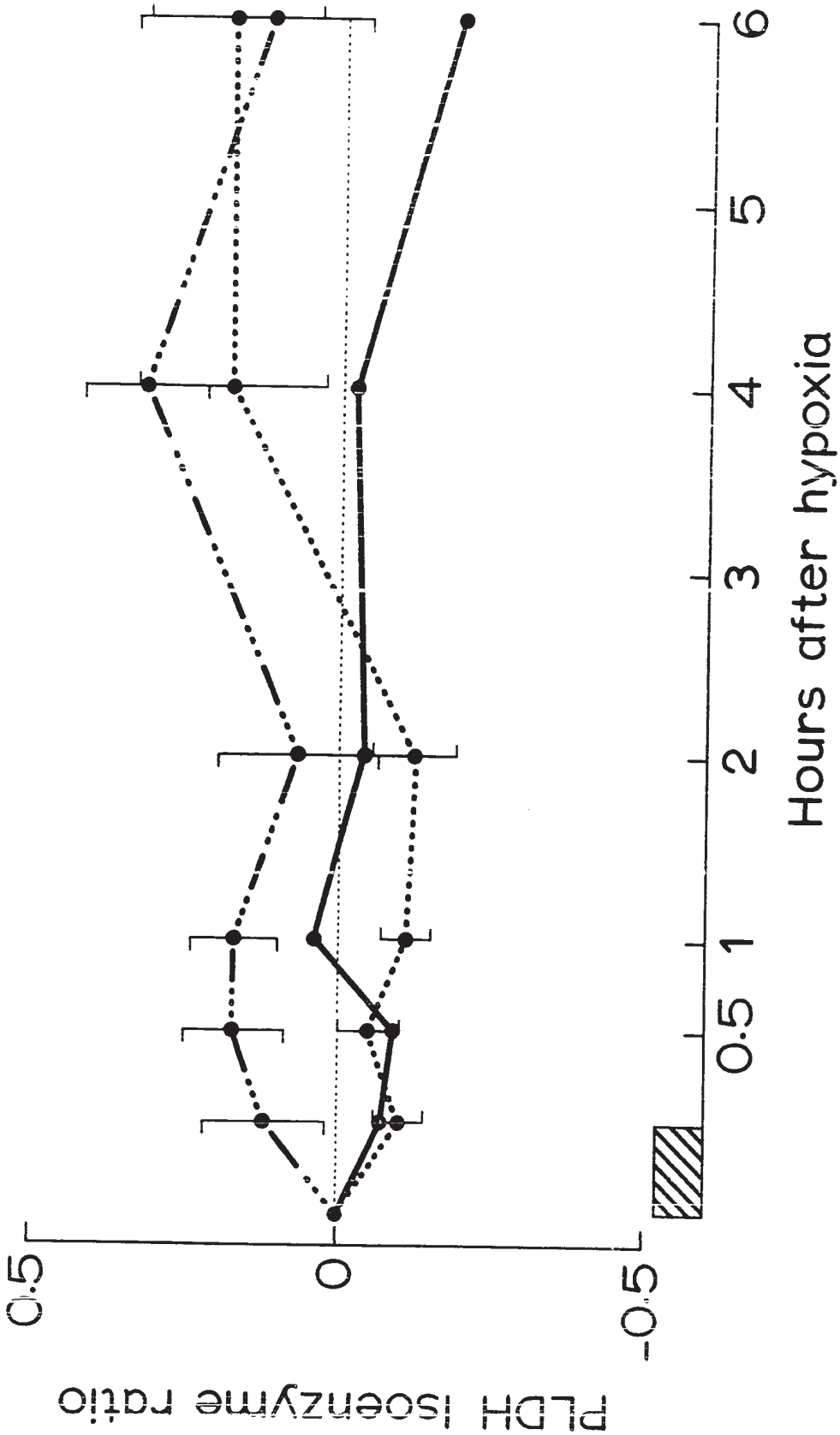
PLDH isoenzyme ratio
0.5
0
-0.5
0.5
1
2
3
4
5
6
Hours after hypoxia

TABLE 30

THE EFFECT OF SUCCINYLCHOLINE ON THE PGOT AND PCPK RESPONSE TO HYPOXIA

Time	PGOT		PCPK	
	10% Oxygen Succinylcholine	10% Oxygen No Succinylcholine	10% Oxygen Succinylcholine	10% Oxygen No Succinylcholine
Before Hypoxia	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
End of Hypoxia	0.7 ± 0.5*	0.8 ± 0.5	3.2 ± 0.6**	3.3 ± 0.9**
1/2 Hour After Hypoxia	0.9 ± 0.5	0.7 ± 0.5	4.7 ± 0.6	4.7 ± 1.9
1 Hour	0.8 ± 0.2	1.3 ± 0.5	7.0 ± 3.5	5.0 ± 0.9
2 Hours	0.3 ± 0.3	0.5 ± 0.4	10.8 ± 4.5	7.3 ± 1.8
4 Hours	-0.2 ± 0.4	0.1 ± 0.9	14.5 ± 4.8	11.1 ± 2.5
6 Hours	-0.9 ± 0.6	0.2 ± 0.6	16.7 ± 5.0	13.3 ± 3.9

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.
** P less than 0.05.

TABLE 31

THE EFFECT OF SUCCINYLCHOLINE ON THE PLDH AND PLDH ISOENZYME RATIO RESPONSE TO HYPOXIA

Time	PLDH		PLDH Isoenzyme Ratio	
	10% Oxygen Succinylcholine	10% Oxygen No Succinylcholine	10% Oxygen Succinylcholine	10% Oxygen No Succinylcholine
Before Hypoxia	0.0 ± 0.0	0.0 ± 0.0	0.00 ± 0.00	00.00 ± 0.00
End of Hypoxia	2.9 ± 1.0**	0.9 ± 0.6*	0.12 ± 0.10	-0.10 ± 0.04
1/2 Hour After Hypoxia	1.4 ± 0.5	1.1 ± 0.6	0.17 ± 0.08	-0.05 ± 0.05
1 Hour	3.9 ± 1.0	1.4 ± 0.7	0.17 ± 0.07	-0.11 ± 0.04
2 Hours	5.5 ± 1.4	1.5 ± 0.4	0.07 ± 0.13	-0.12 ± 0.07
4 Hours	3.2 ± 1.7	2.4 ± 0.8	0.32 ± 0.10	0.18 ± 0.15
6 Hours	2.4 ± 1.5	2.3 ± 0.4	0.14 ± 0.22	0.18 ± 0.14

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.
** P less than 0.05.

TABLE 32

THE EFFECT OF SUCCINYLCHOLINE ON THE ARTERIAL AND VENOUS OXYGEN TENSION RESPONSE TO HYPOXIA

Time	Arterial Oxygen		Venous Oxygen	
	10% Oxygen Succinylcholine	10% Oxygen No Succinylcholine	10% Oxygen Succinylcholine	10% Oxygen No Succinylcholine
Before Hypoxia	61 ± 3*	71 ± 3	44 ± 2	39 ± 3
End of Hypoxia	24 ± 2	22 ± 2	20 ± 1	16 ± 2
1/2 Hour After Hypoxia	74 ± 7	69 ± 2	47 ± 1	41 ± 2
1 Hour	83 ± 7	68 ± 3	49 ± 2	41 ± 2
2 Hours	84 ± 9	71 ± 2	47 ± 3	43 ± 2
4 Hours	85 ± 12	70 ± 3	45 ± 2	41 ± 2
6 Hours	80 ± 9	67 ± 3	42 ± 2	41 ± 3

n is equal to 6.
Oxygen Tension is expressed as mmHg.
* Standard Error of the Mean.

TABLE 33

THE EFFECT OF SUCCINYLCHOLINE ON THE ARTERIAL AND VENOUS CARBON DIOXIDE TENSION RESPONSE TO HYPOXIA

Time	Arterial Carbon Dioxide		Venous Carbon Dioxide	
	10% Oxygen Succinylcholine	10% Oxygen No Succinylcholine	10% Oxygen Succinylcholine	10% Oxygen No Succinylcholine
Before Hypoxia	56 ± 2*	32 ± 3	54 ± 4	37 ± 1
End of Hypoxia	41 ± 2	32 ± 1	43 ± 2	36 ± 1
1/2 Hour After Hypoxia	38 ± 5	35 ± 1	41 ± 5	40 ± 2
1 Hour	37 ± 4	37 ± 2	42 ± 5	42 ± 2
2 Hours	46 ± 4	39 ± 3	48 ± 4	43 ± 3
4 Hours	49 ± 5	38 ± 2	51 ± 5	43 ± 2
6 Hours	46 ± 5	38 ± 2	48 ± 5	43 ± 3

n is equal to 6.
Carbon dioxide tension is expressed as mmHg.
* Standard Error of the Mean.

TABLE 34

THE EFFECT OF SUCCINYLCHOLINE ON THE ARTERIAL SYSTOLIC AND DIASTOLIC BLOOD PRESSURE AND HEART RATE RESPONSE TO HYPOXIA

Time	Systolic Blood Pressure		Diastolic Blood Pressure		Heart Rate	
	10% Oxygen Succinyl-choline	10% Oxygen No Succinyl-choline	10% Oxygen Succinyl-choline	10% Oxygen No Succinyl-choline	10% Oxygen Succinyl-choline	10% Oxygen No Succinyl-choline
Before Hypoxia	134 ± 8*	139 ± 9	108 ± 7	115 ± 9	173 ± 10	163 ± 10
Peak Blood Pressure	161 ± 8 (10 min.)***	184 ± 11 (7 min.)	127 ± 10 (10 min.)	146 ± 8 (7 min.)	174 ± 14 (10 min.)	171 ± 12 (7 min.)
End of Hypoxia	129 ± 16	164 ± 10	108 ± 14	130 ± 8	168 ± 13	161 ± 6
1/2 Hour After Hypoxia	125 ± 13	123 ± 9	105 ± 10	100 ± 7	171 ± 10	149 ± 13
1 Hour	128 ± 10	133 ± 6	106 ± 10	107 ± 6	167 ± 8	146 ± 10
2 Hours	137 ± 10	129 ± 9	116 ± 9	106 ± 8	163 ± 7	148 ± 9
4 Hours	142 ± 9	132 ± 9	117 ± 9	108 ± 8	152 ± 12	149 ± 11
6 Hours	146 ± 12	133 ± 10	122 ± 10	107 ± 10	159 ± 14	154 ± 14

n is equal to 6.
Blood Pressure is expressed as mmHg.
Heart Rate is expressed as beats per minute.
* Standard Error of the Mean
*** Time after beginning of hypoxia when peak blood pressure was reached.

V. DISCUSSION

(A) Hypoxia

The purpose of this series of experiments was to find the level of hypoxia that would cause a release of enzymes into the blood. The threshold for PGOT was higher than that for PCPK and PLDH. The threshold for GOT release was between 7.5 and 8.5 percent oxygen in the inspired air which produced arterial oxygen tensions of 11 and 17 mmHg respectively. CPK and LDH had similar thresholds for release which were between 10 and 12 percent oxygen for 24 and 31 mmHg arterial oxygen tension.

The thresholds for PGOT and PLDH may be compared to those reported previously, however, the threshold for PCPK has not been previously studied. Highman and Altland (1966) found that rats exposed to a simulated altitude of 22,000 feet (321 mmHg) for five hours showed an increase in PLDH but not in PGOT. Rats exposed to 25,000 feet (282 mmHg) had an increase in both PGOT and PLDH while exposure to 18,000 feet (379 mmHg) caused no change in the plasma activity of these enzymes. Highman and Altland found the threshold for PGOT between 25,000 and 22,000 feet altitude or between 49 and 58 mmHg in the inspired air. The present study found this threshold between 7.5 and

8.5 percent oxygen (54 and 61 mmHg oxygen) in the inspired air. Similarly, Highman and Altland found the threshold for PLDH between 22,000 and 18,000 feet altitude or between 58 and 70 mmHg oxygen in the inspired air. The present study found this threshold to be between 10 and 12 percent oxygen (71 and 86 mmHg oxygen) in the inspired air. Thus, the thresholds for these two enzymes is slightly lower in this study than that reported by Highman and Altland. This difference is probably due to the fact that although their exposure was of much greater duration, the rats were allowed to hyperventilate during the exposure which probably resulted in higher blood oxygen tensions.

Refsum (1963), studying patients with pulmonary insufficiency, observed an increase in PLDH when the oxygen content of arterial blood fell below 12 volumes percent (31 mmHg) and an increase in both PGOT and PLDH when the oxygen content was below nine volumes percent (24 mmHg). This threshold for PLDH agrees very well with the present study which found the threshold between 31 and 24 mmHg oxygen tension in the arterial blood. Refsum, however, reported large increases in PGOT with arterial oxygen tensions of 22 mmHg whereas the present study did not find an increase in PGOT until the arterial oxygen tension was below 17 mmHg. In the study published by Refsum it is very difficult to determine the duration of the various levels of hypoxia.

It is well known that catecholamines are released from the

adrenal medulla at an increased rate during hypoxia (Hatcher and Jennings, 1966). It has also been shown that infusions of either adrenaline or noradrenaline will cause an increase in plasma enzyme activity (Loegering and Critz, 1968; Highman, Maling and Thompson, 1959). Fowler, Shabetae and Holmes (1961) found that catecholamines were released from the adrenal glands of dogs at a rate of 0.3 microgram per kilogram per minute while being ventilated with seven percent oxygen. The animals in this study had an arterial oxygen tension of about 28 mmHg during the hypoxia. Later, Binnion and Hatcher (1963) found that infusing adrenaline at a rate of 0.2 microgram per kilogram per minute produced cardiac output changes similar to those seen in the level of hypoxia studied by Fowler et al (1961). Loegering and Critz (1968) found that infusions of noradrenaline in dogs at a rate of 1.6 micrograms per kilogram per minute was the smallest amount of noradrenaline that would cause an increase in PGOT activity. Highman, Maling and Thompson (1959) found that adrenaline and noradrenaline are equally potent in terms of causing an increase in PGOT when infused into dogs. Severe levels of hypoxia cause large increases in the blood levels of catecholamines (Ludemann, Filbert and Cornblath, 1956). Several studies have shown that at more severe levels of hypoxia the pressor response to catecholamines is greatly reduced (Van Loo, Surshin and Katz, 1948; Duner and Euler, 1959; Cowdey, 1966). It can be concluded from these results that catecholamines

released in response to hypoxia do not play a role in the enzyme response to hypoxia. The increase in plasma levels of catacholamines during exercise is less than that seen in hypoxia (Vendsalu, 1960) so it is very likely that catecholamines do not affect the enzyme response to exercise (Loegering and Critz, 1968).

The possibility that the increase in heart rate and blood pressure during exercise may be contributing to the plasma enzyme response to exercise is currently being studied in our laboratory. Pacing the heart of anesthetized dogs at a rate of 240 beats per minute for 30 minutes causes no change in PCPK or PGOT (Bolter and Critz, 1969). Stimulation of the Fields of Forel in the posterior hypothalamus causes changes in blood pressure and heart rate similar to those seen in exercise (Rushmer, Smith and Franklin, 1959). Preliminary results of similar experiments performed in our laboratory have indicated that an increase in blood pressure in the absence of cardiac acceleration will not cause a change in plasma enzyme activity (Bolter and Critz, 1969). An increase in both blood pressure and heart rate for 30 minutes (heart rate over 200 beats per minute and systolic blood pressure over 190 mmHg) will cause an increase in PCPK and PGOT (Bolter and Critz, 1969). The magnitude of the changes in blood pressure and heart rate required to cause an increase in plasma enzyme activity indicates that in the present study such changes are probably not responsible for the release of enzymes. The increase in blood

pressure and heart rate may contribute to the plasma enzyme response to exercise.

There is essentially no change in the oxygen content of the arterial blood during exercise. There is a decrease in the oxygen content of the central venous blood which can be related to the intensity of the exercise (Rowell, 1969). During maximal exercise the arteriovenous oxygen difference may reach 17 volumes percent (Saltin, 1969) which would result in an oxygen tension in the central venous blood of about 10 mmHg. The femoral vein oxygen content during maximal exercise is one or two volumes percent less than the oxygen content of central venous blood (Saltin, Blomqvist, Mitchell, Johnson, Windenthal and Chapman, 1968). A work load causing a heart rate of 150 beats per minute will decrease the central venous oxygen tension from a resting value of 50 mmHg to 25 mmHg (Donald, Bishop, Cumming and Wade, 1955). At the same work load the femoral vein oxygen tension will decrease to 15 mmHg (Reeves, Grover, Filley and Blount, 1961). In the present study, ventilating animals with 12, 10, 8.5 or 7.5 percent oxygen resulted in venous oxygen tensions of 23, 20, 9 or 6 mmHg respectively. Since 7.5 percent oxygen caused an increase in PGOT, PCPK and PLDH, this level of hypoxia may be responsible for the increase in plasma enzyme activity with maximal exercise. However, exercising at a heart rate of 150 beats per minute will also cause an increase in PGOT, PCPK and PLDH (Hunter and Critz, 1969). Since 8.5 percent

oxygen caused an increase in PCPK and PLDH but not in PGOT it is likely that the PGOT response to submaximal exercise is not caused by hypoxia.

It is interesting to point out that long term exercise such as walking (Griffiths, 1966) or marching (Nerdrum and Berg, 1964; Halonen and Konttinen, 1962) caused an increase in PCPK and PLDH while PGOT remained unchanged. Reeves et al (1961) found that exercising at a heart rate of 100 beats per minute (heart rate associated with walking or marching) resulted in a femoral venous oxygen tension of 21 mmHg. This oxygen tension is close to that resulting from 10 percent oxygen (20 mmHg venous oxygen tension) which caused an increase in PCPK and PLDH but did not alter PGOT activity. These data indicate that the PCPK and PLDH response to exercise may be caused by hypoxia.

The ability of physical training to reduce the plasma enzyme response to exercise must be considered in any discussion of the mechanism of enzyme release during exercise. Holmgren, Mossfeldt, Sjöstrand and Stram (1960) found that training causes a more efficient peripheral circulatory adaptation to muscular work. In 1962, Elsner and Carlson found that trained subjects had greater blood flow in exercising muscles than untrained subjects during similar submaximal work loads. In the same study it was shown that untrained subjects have a relative ischemia in exercising muscles as indicated by post-

exercise hyperemia. Bed rest (20 days) resulted in a greater arterio-venous oxygen difference during submaximal exercise (Saltin et al. 1968). These results indicate that training results in an increased blood supply to exercising muscles which would reduce the degree of hypoxia in the muscles.

Physical training may reduce the degree of muscle hypoxia during submaximal exercise, however, at maximal levels of exercise such a decrease could not occur. In fact, Saltin et al (1968) found that trained subjects have a greater central arterio-venous oxygen difference during maximal exercise. Hunter and Critz (1969) have shown that training will reduce the plasma enzyme response to maximal exercise. This indicates that some mechanism other than a reduction in hypoxia is responsible for the decreased plasma enzyme response to exercise associated with training. Highman and Altland (1963) found that rats exercising for six hours per day showed a progressive increase in PGOT and PLDH during the first three days. This was followed by a progressive decrease in enzyme response until at seven days there was no increase in these enzymes after exercise. Histologic examination of the muscles of these animals revealed a correlation between muscle necrosis and the enzyme response. After three days of training 63 percent of the animals had necrotic areas in skeletal muscles; after eight days of training only five percent of the animals demonstrated such changes. This information indicates that

training may improve the tolerance to exercise at the cellular level, and that this may be the mechanism for the reduction in the enzyme response to exercise after training.

(B) Simulated Exercise

Stimulation of the hind leg muscles of the dog at five pulses per second for 30 minutes did not change the plasma enzyme activity. Stimulation at 10 pulses per second resulted in an increase in PGOT and PLDH activity. The lack of a change in the PLDH isoenzyme ratio is consistent with other studies on exercise (Garbus et al, 1964; Papadopoulos et al, 1967; 1968). This type of response indicates that LDH is released from several different tissues during exercise.

At the end of stimulation, both groups had the same femoral venous oxygen tension, and the oxygen consumption of the two groups was not significantly different. Kjellmer (1964) found little difference in blood flow through the calf muscles of cats when stimulated at a rate of five and 10 pulses per second and the highest rate of blood flow was found at stimulation rates of eight per second. It has been reported that the oxygen consumption of an isolated muscle does not increase when the stimulation rate is increased above five per second (Stainsby, 1966). Subsequent studies on two animals showed that after stimulation at a rate of 10 pulses per second there was an immediate increase in carbon dioxide tension in femoral venous blood which reached a peak of 60 mmHg about 15 seconds after the stimulation.

Similarly, there was an increase in oxygen tension which reached a peak of 45 mmHg one minute after the stimulation. This was higher than the pre-stimulation oxygen tension in these experiments. No increase in femoral vein blood gas tensions were found following stimulation at five pulses per second. These changes are very similar to those seen in reactive hyperemia (Kontos and Patterson, 1964) and after sustained contraction of limb muscles (Barcroft, Greenwood and Whelan, 1963). Such results indicate that stimulation at 10 pulses per second was producing a greater ischemia in the muscles than stimulation at five pulses per second.

Although PCPK and PLDH are equally sensitive to hypoxia, there was no increase in PCPK after the simulated exercise. The response of PLDH to simulated exercise was a very sharp increase followed by a rapid return toward normal which is quite different from the more slowly rising and longer lasting response of this enzyme to hypoxia. Considering these results one must conclude that hypoxia is not the only factor that is responsible for the plasma enzyme response to exercise.

At this point, the possibility that blood carbon dioxide tension or pH may be influencing the plasma enzyme response to exercise must be considered. Jacey and Schaefer (1967) found that guinea pigs placed in an atmosphere of 15 percent carbon dioxide and 21 percent oxygen showed no change in PLDH activity after six hours at which time the

arterial blood pH was 7.04. After 24 hours of exposure to 15 percent carbon dioxide the PLDH activity had doubled and the arterial pH was 7.09. In 1958, Mitchell, Sproule and Chapman found that during maximal exercise the femoral venous blood pH was 7.13, however, two minutes after the exercise the pH decreased to 7.01 and 10 minutes after the exercise it had returned to 7.11. In the same study, the femoral vein carbon dioxide tension was 83 mmHg during the exercise and was 82 mmHg two minutes after the exercise. It is also interesting to note that Saltin et al (1968) has shown that trained subjects have lower femoral venous blood pH and higher carbon dioxide tensions than untrained subjects during maximal exercise. The extremely slow response of PLDH to lowered blood pH and the magnitude of pH and carbon dioxide changes during exercise suggests that these changes do not play a role in the plasma enzyme response to exercise.

(C) Multiple Sampling Sites

There was no difference in plasma enzyme activity in blood taken simultaneously from four sampling sites (carotid artery, femoral vein, inferior vena cava and right atrium) in animals ventilated with five percent oxygen. This indicates that five percent oxygen causes similar release of enzymes from many tissues of the body. This finding is verified for PGOT and PCPK in the experiments with 10 percent oxygen. The PLDH activity of arterial blood was higher

than in the blood taken from the right atrium in the 10 percent oxygen group. This difference occurred during the first two hours after hypoxia and was not evident at four and six hours after the hypoxia. At this level of hypoxia and during the first two to four hours after hypoxia, there may be a greater release of LDH from the heart than from other tissues. This suggestion is not verified by the PLDH isoenzyme ratio which showed no change in the proportion of M and H subunit activity. In 1959, Vesell, Feldman and Frank found higher PLDH activity in venous blood than in arterial blood during hemorrhagic shock in dogs. These authors also showed there was no change in the proportions of PLDH isoenzymes during hemorrhagic shock. Similar results have been obtained with hypoxia (Altland, Highman and Garbus, 1964; Highman and Altland, 1966; Selmeci et al, 1967).

The threshold for GOT release coincides with the threshold for ischemic ECG changes which may suggest that GOT is released from the heart during hypoxia. This is in agreement with the suggestion that LDH is released from the heart in proportionately greater amounts than from other tissues with 10 percent oxygen. The absence of a significant difference in enzyme activity in samples taken from different sampling sites in the group ventilated with five percent oxygen does not support this possibility. It may be that the heart is the tissue most sensitive to hypoxia in terms of enzyme release; then as the threshold of hypoxia for a particular enzyme is approached the release

of that enzyme will be primarily from the heart. There is no data to support this supposition for the release of CPK.

(D) Prednisolone and Nitroglycerin

The drug control group which received both prednisolone and nitroglycerin and was ventilated with room air for 30 minutes showed no change in plasma enzyme activity. These results indicate that in the absence of hypoxia neither of the drugs were affecting the plasma enzyme levels.

Prednisolone did not alter the PCPK, PLDH or PLDH isoenzyme ratio response to 7.5 percent oxygen. PGOT did not increase when compared with the drug control group. The arterial oxygen tension in the prednisolone-treated group was higher at the end of the hypoxia (17 mmHg) than in the non-treated group ventilated with 7.5 percent oxygen (11 mmHg). The arterial oxygen tension in the prednisolone-treated group was similar to that of the group ventilated with 8.5 percent oxygen. The lack of an increase in PGOT activity is probably due to the less severe hypoxia in the prednisolone-treated group, rather than to an effect of prednisolone.

Nitroglycerin did not alter the PCPK response to 7.5 percent oxygen, however, PGOT and PLDH did not increase when compared with the drug control group. The PLDH isoenzyme ratio increased in the animals given nitroglycerin while being ventilated with 7.5 percent oxygen. This indicates that there was a proportionately greater release

of LDH from skeletal muscle and liver tissue than from heart tissue.

The arterial oxygen tension in the nitroglycerin-treated group was higher at the end of the hypoxia (17 mmHg) than in the non-treated group ventilated with 7.5 percent oxygen (11 mmHg). The arterial oxygen tension in the nitroglycerin-treated group was similar to that of the group ventilated with 8.5 percent oxygen. The lack of an increase in PGOT is probably due to the less severe hypoxia in the nitroglycerin-treated group rather than to an effect of nitroglycerin.

Since the PLDH response to 7.5 and 8.5 percent oxygen is similar, the lack of an increase in PLDH activity observed in the nitroglycerin-treated group must be due to an effect of nitroglycerin. The reduction in the PLDH response to hypoxia caused by nitroglycerin is probably due to a reduction in the amount of this enzyme released from the heart as indicated by the PLDH isoenzyme ratio. The ability of nitroglycerin to reduce myocardial oxygen consumption (Bernstein et al, 1966) is probably responsible for the decreased LDH release from the heart during hypoxia.

Previous studies have shown that prednisolone is capable of reducing the PCPK response to exercise (Wagner and Critz, 1968) and that nitroglycerin is capable of reducing the PGOT and PCPK response to exercise (Nerdrum and Nordøy, 1964; Wagner, 1967). The inability of these drugs to decrease the PGOT and PCPK response to hypoxia indicates that the mechanism of release of these enzymes

during hypoxia and exercise is different. Although nitroglycerin reduced the PLDH response to hypoxia, the data from the simulated exercise and hypoxia studies suggests that hypoxia is not the major cause of the release of this enzyme during exercise.

(E) Succinylcholine

The administration of succinylcholine during hypoxia had no effect on the plasma enzyme response. Tammisto and Airaksinen (1966) and Tammisto, Leikkonen and Airaksinen (1967) found that repeated injections of succinylcholine (one milligram per kilogram or 0.5 milligram per kilogram) in patients caused an increase in PCPK. Adult patients show a much smaller increase in PCPK after injections of succinylcholine than children (Tammisto, Brander and Airaksinen, 1969). The control animals in the pre sent study received succinylcholine and there was no increase in plasma enzyme activity. The group that did not receive succinylcholine and was ventilated with 10 percent oxygen showed the same enzyme response to hypoxia as the group receiving succinylcholine while being ventilated with 10 percent oxygen. The lack of an effect by succinylcholine on the plasma enzyme activity in the present study may be due to the fact that adult animals were used and that the dose of succinylcholine was about one-half that used by Tammisto and Airaksinen (1966) and Tammisto, Leikkonen and Airaksinen (1967).

(F) General Discussion

This study was designed to determine if the relative hypoxia that exists in exercising muscles is responsible for the plasma enzyme response to exercise. The level of hypoxia causing a release of enzymes into the blood was determined by ventilating animals with gas mixtures containing known amounts of oxygen. The results indicate that the threshold for GOT release was between the level of hypoxia produced by 7.5 and 8.5 percent oxygen (11 mmHg and 17 mmHg arterial oxygen tension). The enzymes, CPK and LDH, had thresholds for release between the level of hypoxia produced by 10 and 12 percent oxygen (24 mmHg and 31 mmHg arterial oxygen tension).

Comparison of the blood oxygen tensions observed during exercise with those associated with a release of enzymes into the blood indicate that in untrained individuals hypoxia may cause the changes in plasma enzyme activity associated with maximal exercise. The increase in PGOT activity with submaximal exercise is probably not due to hypoxia. Physical training reduces the plasma enzyme response to exercise (Hunter and Critz, 1969) and improves the blood supply to exercising muscles (Elsner and Carlson, 1962). During maximal exercise in trained subjects the level of hypoxia in the exercising muscles is not reduced (Saltin et al, 1968), thus, the ability of training to reduce the plasma enzyme response to exercise may not be due to a reduction in the hypoxia in exercising muscles.

A second series of experiments were performed to determine

the effect of simulated exercise on plasma enzyme activity. These experiments were carried out so that plasma enzyme and blood gas changes during hypoxia and exercise could be compared under similar experimental conditions. Simulated exercise caused an increase in PGOT and PLDH activity but there was no change in PCPK activity. Although PCPK and PLDH are equally sensitive to hypoxia there was no increase in PCPK after simulated exercise. PGOT and PLDH showed a much more transient response to simulated exercise than to hypoxia. These results indicate that hypoxia is not the major factor responsible for the plasma enzyme response to exercise.

Previous studies have shown that prednisolone and nitroglycerin are capable of reducing the PGOT and PCPK response to exercise (Nerdrum and Nordøy, 1964; Wagner and Critz, 1968; Wagner, 1967). The inability of these drugs to reduce the response of these enzymes to hypoxia suggests that the mechanism of enzyme release during exercise and hypoxia is different.

Several factors that may be responsible for the plasma enzyme response to exercise were considered. Data from other studies indicate that changes in blood carbon dioxide tension and pH during exercise (Jacey and Schaefer, 1967) as well as the release of catecholamines (Loegering and Critz, 1968) are not responsible for the plasma enzyme response to exercise. Changes in blood pressure and heart rate during hypoxia have no effect, but the much greater changes in these parameters during exercise may contribute to the plasma enzyme

response to exercise (Bolter and Critz, 1969).

VI. SUMMARY AND CONCLUSIONS

This study was designed to determine if the relative hypoxia that exists in exercising muscles is responsible for the plasma enzyme response to exercise. The role of hypoxia in the plasma enzyme response to exercise was determined by finding the degree of hypoxia required to cause a release of enzymes. A second series of experiments determined the plasma enzyme response to simulated exercise (muscle stimulation) which simulated the hypoxia that exists in exercising muscles. Further experiments were carried out to determine if drugs (prednisolone and nitroglycerin) that are capable of decreasing the plasma enzyme response to exercise will decrease the plasma enzyme response to hypoxia. All experiments were performed on anesthetized dogs. These experiments showed that:

(1) The threshold for GOT release was between the level of hypoxia produced by 7.5 and 8.5 percent oxygen (11 mmHg and 17 mmHg arterial oxygen tension). The enzymes, CPK and LDH, had thresholds for release between the level of hypoxia produced by 10 and 12 percent oxygen (24 mmHg and 31 mmHg arterial oxygen tension). Hypoxia caused no change in the PLDH isoenzyme ratio which indicates that this enzyme

is released from several tissues.

(2) Plasma enzyme activity of samples taken simultaneously from various sampling sites gave no clear indication of the source of the enzymes released during and following hypoxia. It was concluded from these experiments that hypoxia causes a release of enzymes from several tissues.

(3) Electrical stimulation of the muscles of the hind legs of anesthetized animals at a rate of five pulses per second caused no change in the plasma enzyme levels. Stimulation at 10 pulses per second caused an increase in PGOT and PLDH but there was no change in PCPK activity. PGOT and PLDH showed a much more transient response to simulated exercise than to hypoxia. There was no change in the PLDH isoenzyme ratio which indicates that there was a release of this enzyme from skeletal muscle, heart and possibly other tissues during muscle stimulation.

(4) Prednisolone did not alter the plasma enzyme response to hypoxia. Nitroglycerin reduced the PLDH response but did not alter the PGOT and PCPK response to hypoxia. Animals receiving nitroglycerin during hypoxia showed an increase in PLDH isoenzyme ratio which indicates that there was proportionately more of this enzyme released from skeletal muscle or liver than from heart.

It was concluded from this study that hypoxia is not the major cause of the plasma enzyme response to exercise.

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APPENDIX A

Means of Raw Enzyme Data: Plasma Enzyme Response to Various Levels of Hypoxia

TABLE 35

PGOT RESPONSE TO VARIOUS LEVELS OF HYPOXIA

Time	Control (30 min.)	12 Percent Oxygen (30 min.)	10 Percent Oxygen (30 min.)	8.5 Percent Oxygen (30 min.)	7.5 Percent Oxygen (30 min.)	5 Percent Oxygen (15 min.)
Before Hypoxia	10.7 ± 0.5*	5.9 ± 0.7	11.3 ± 0.9	12.5 ± 2.6	8.1 ± 1.4	10.3 ± 1.1
End of Hypoxia	10.6 ± 0.3	6.7 ± 0.7	11.9 ± 1.2	15.1 ± 3.4	9.1 ± 0.9	13.0 ± 1.2
1/2 Hour After Hypoxia	10.3 ± 0.3	6.6 ± 0,9	12.2 ± 1.1	15.1 ± 3.6	10.3 ± 0.8	16.1 ± 1.6
1 Hour	11.0 ± 0.5	6.3 ± 0.8	12.0 ± 0.8	14.4 ± 3.7	10.0 ± 1.1	15.9 ± 1.1
2 Hours	10.6 ± 0.7	6.1 ± 0.3	11.5 ± 0.9	13.8 ± 4.1	11.2 ± 0.8	16.3 ± 1.6
4 Hours	9.8 ± 0.6	5.5 ± 0.5	11.1 ± 0.6	12.2 ± 2.6	10.7 ± 0.8	17.3 ± 1.6
6 Hours	9.2 ± 0.7	5.4 ± 0.4	10.4 ± 0.5	11.2 ± 2.3	10.7 ± 0.9	14.7 ± 2.2

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

TABLE 36

PCPK RESPONSE TO VARIOUS LEVELS OF HYPOXIA

Time	Control (30 min.)	12 Percent Oxygen (30 min.)	10 Percent Oxygen (30 min.)	8.5 Percent Oxygen (30 min.)	7.5 Percent Oxygen (30 min.)	5 Percent Oxygen (15 min.)
Before Hypoxia	34.8 ± 3.8*	12.0 ± 3.1	14.2 ± 2.0	14.7 ± 8.6	10.8 ± 1.0	14.5 ± 3.3
End of Hypoxia	32.3 ± 4.1	12.0 ± 3.1	17.2 ± 1.7	16.3 ± 4.4	14.3 ± 2.0	17.2 ± 4.9
1/2 Hour After Hypoxia	34.8 ± 4.9	12.2 ± 3.0	18.7 ± 2.1	18.3 ± 3.8	19.5 ± 2.1	24.8 ± 4.2
1 Hour	34.5 ± 5.6	13.2 ± 3.2	21.2 ± 4.9	21.5 ± 4.2	23.8 ± 3.3	32.5 ± 7.0
2 Hours	34.8 ± 5.0	14.5 ± 3.0	25.0 ± 5.7	20.5 ± 3.8	27.8 ± 4.0	34.0 ± 8.3
4 Hours	32.7 ± 3.0	14.0 ± 2.5	28.7 ± 5.9	22.0 ± 3.3	30.5 ± 6.0	41.2 ± 7.9
6 Hours	33.3 ± 3.7	14.0 ± 3.5	30.8 ± 5.5	21.8 ± 4.0	32.0 ± 4.5	41.5 ± 7.2

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

TABLE 37

PLDH RESPONSE TO VARIOUS LEVELS OF HYPOXIA

Time	Control (30 min.)	12 Percent Oxygen (30 min.)	10 Percent Oxygen (30 min.)	8.5 Percent Oxygen (30 min.)	7.5 Percent Oxygen (30 min.)	5 Percent Oxygen (15 min.)
Before Hypoxia	11.4 ± 3.0*	3.0 ± 0.3	9.3 ± 0.7	3.1 ± 0.5	6.9 ± 1.7	8.0 ± 1.6
End of Hypoxia	11.7 ± 2.9	3.6 ± 0.4	12.2 ± 1.2	4.3 ± 1.0	10.7 ± 1.6	11.5 ± 2.1
1/2 Hour After Hypoxia	11.4 ± 2.6	3.6 ± 0.4	10.6 ± 0.7	5.7 ± 1.3	9.7 ± 1.8	16.0 ± 2.6
1 Hour	12.1 ± 2.7	3.4 ± 0.4	13.1 ± 1.0	7.2 ± 1.0	9.7 ± 1.3	17.3 ± 2.9
2 Hours	11.4 ± 2.7	3.4 ± 0.4	14.7 ± 1.2	5.8 ± 1.4	11.4 ± 1.6	19.0 ± 3.6
4 Hours	11.8 ± 3.2	3.8 ± 0.5	12.5 ± 1.4	7.0 ± 1.0	12.4 ± 1.0	20.0 ± 2.8
6 Hours	11.8 ± 2.9	3.5 ± 0.5	11.6 ± 1.4	6.1 ± 1.1	10.3 ± 0.8	17.8 ± 2.0

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

TABLE 38

PLDH ISOENZYME RATIO RESPONSE TO VARIOUS LEVELS OF HYPOXIA

Time	Control (30 min.)	12 Percent Oxygen (30 min.)	10 Percent Oxygen (30 min.)	8.5 Percent Oxygen (30 min.)	7.5 Percent Oxygen (30 min.)	5 Percent Oxygen (30 min.)
Before Hypoxia	3.19 ± 0.11*	2.75 ± 0.12	3.03 ± 0.12	2.91 ± 0.11	2.76 ± 0.10	3.05 ± 0.14
End of Hypoxia	3.12 ± 0.08	2.80 ± 0.16	3.13 ± 0.12	2.90 ± 0.15	2.66 ± 0.17	2.86 ± 0.16
1/2 Hour After Hypoxia	3.11 ± 0.11	2.74 ± 0.10	3.20 ± 0.15	3.06 ± 0.13	2.57 ± 0.12	3.13 ± 0.19
1 Hour	3.22 ± 0.09	2.77 ± 0.10	3.30 ± 0.13	3.09 ± 0.12	2.72 ± 0.18	3.03 ± 0.19
2 Hours	3.15 ± 0.05	2.77 ± 0.14	3.09 ± 0.16	3.08 ± 0.15	2.89 ± 0.21	3.03 ± 0.26
4 Hours	3.16 ± 0.09	2.77 ± 0.05	3.35 ± 0.13	2.80 ± 0.21	2.88 ± 0.11	3.04 ± 0.27
6 Hours	2.99 ± 0.07	2.76 ± 0.06	3.16 ± 0.19	2.85 ± 0.16	2.82 ± 0.15	3.05 ± 0.17

n is equal to 6.
*Standard Error of the Mean.

APPENDIX B

Means of Raw Enzyme Data: Plasma Enzyme Response to Simulated Exercise

TABLE 39

PGOT AND PCPK RESPONSE TO SIMULATED EXERCISE

Time	PGOT		PCPK	
	5 Pulses/Sec. (30 min.)	10 Pulses/Sec. (30 min.)	5 Pulses/Sec. (30 min.)	10 Pulses/Sec. (30 min.)
Before Stimulation	9.4 ± 1.3*	8.1 ± 1.4	21.5 ± 5.7	15.3 ± 3.3
End of Stimulation	12.0 ± 2.2	11.6 ± 2.0	23.0 ± 5.7	16.3 ± 3.5
1/2 Hour After Stimulation	12.0 ± 1.6	11.5 ± 1.8	25.3 ± 6.9	18.8 ± 3.9
1 Hour	10.8 ± 1.0	11.7 ± 1.5	25.8 ± 8.2	17.2 ± 3.4
2 Hours	10.4 ± 1.3	9.7 ± 1.6	27.5 ± 9.8	19.0 ± 3.3
4 Hours	9.6 ± 1.0	8.3 ± 1.4	26.5 ± 9.5	20.7 ± 3.8
6 Hours	8.1 ± 1.0	8.3 ± 1.5	23.7 ± 5.0	19.7 ± 4.3

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

TABLE 40

PLDH AND PLDH ISOENZYME RATIO RESPONSE TO SIMULATED EXERCISE

Time	PLDH		PLDH Isoenzyme Ratio	
	5 Pulses/Sec. (30 min.)	10 Pulses/Sec. (30 min.)	5 Pulses/Sec. (30 min.)	10 Pulses/Sec. (30 min.)
Before Stimulation	5.6 ± 0.7*	4.7 ± 1.0	2.69 ± 0.13	2.72 ± 0.08
End of Stimulation	7.8 ± 2.0	16.2 ± 4.2	2.64 ± 0.09	2.82 ± 0.08
1/2 Hour After Stimulation	7.8 ± 1.8	14.5 ± 4.0	2.59 ± 0.12	2.93 ± 0.14
1 Hour	8.0 ± 1.9	11.8 ± 3.1	2.63 ± 0.11	2.84 ± 0.12
2 Hours	8.5 ± 1.2	9.8 ± 2.2	2.64 ± 0.12	2.86 ± 0.10
4 Hours	8.0 ± 1.3	7.0 ± 1.1	2.81 ± 0.10	2.81 ± 0.09
6 Hours	7.1 ± 1.4	5.7 ± 0.7	2.92 ± 0.11	2.81 ± 0.10

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

APPENDIX C

Means of Raw Enzyme Data: Plasma Enzyme Activity of Simultaneous Samples from Different Sampling Sites

TABLE 41

PGOT ACTIVITY OF SAMPLES TAKEN SIMULTANEOUSLY FROM FOUR SAMPLING SITES IN ANIMALS RESPIRED WITH FIVE PERCENT OXYGEN FOR 15 MINUTES

Time	Carotid Artery	Right Atrium	Femoral Vein	Inferior Vena Cava
Before Hypoxia	11.0 ± 1.1*	11.4 ± 1.1	11.1 ± 1.1	10.1 ± 1.0
End of Hypoxia	13.7 ± 1.2	13.1 ± 0.8	13.7 ± 0.7	13.4 ± 0.9
1/2 Hour After Hypoxia	17.2 ± 1.4	18.2 ± 1.2	17.1 ± 1.3	16.0 ± 0.8
1 Hour	16.7 ± 0.6	17.6 ± 1.1	16.7 ± 0.8	17.1 ± 0.6
2 Hours	17.7 ± 0.9	17.8 ± 1.3	17.6 ± 1.0	18.6 ± 1.3
4 Hours	18.0 ± 1.8	18.1 ± 1.9	16.4 ± 1.7	18.1 ± 2.1
6 Hours	16.0 ± 2.2	17.2 ± 1.7	15.5 ± 1.2	17.6 ± 1.9

n is equal to 5.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

TABLE 42

PCPK ACTIVITY OF SAMPLES TAKEN SIMULTANEOUSLY FROM FOUR SAMPLING SITES IN ANIMALS RESPIRED WITH FIVE PERCENT OXYGEN FOR 15 MINUTES

Time	Carotid Artery	Right Atrium	Femoral Vein	Inferior Vena Cava
Before Hypoxia	16.0 ± 3.6*	19.4 ± 4.0	16.6 ± 4.0	16.0 ± 3.5
End of Hypoxia	19.4 ± 5.0	20.4 ± 3.9	20.6 ± 4.8	19.0 ± 3.6
1/2 Hour After Hypoxia	25.2 ± 5.1	29.2 ± 5.3	29.6 ± 6.1	25.8 ± 5.0
1 Hour	34.4 ± 8.2	32.2 ± 7.2	34.4 ± 7.6	32.6 ± 7.9
2 Hours	36.8 ± 9.6	37.4 ± 8.8	40.8 ± 8.3	38.4 ± 7.9
4 Hours	45.2 ± 8.3	44.8 ± 11.3	45.0 ± 9.0	41.8 ± 8.4
6 Hours	45.8 ± 7.0	51.0 ± 8.7	51.4 ± 10.5	48.4 ± 8.2

n is equal to 5.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

TABLE 43

PLDH ACTIVITY OF SAMPLES TAKEN SIMULTANEOUSLY FROM FOUR SAMPLING SITES IN ANIMALS RESPIRED WITH FIVE PERCENT OXYGEN FOR 15 MINUTES

Time	Carotid Artery	Right Atrium	Femoral Vein	Inferior Vena Cava
Before Hypoxia	8.7 ± 1.7*	10.8 ± 2.2	8.9 ± 1.3	9.3 ± 1.0
End of Hypoxia	12.0 ± 2.5	13.4 ± 3.2	16.3 ± 4.7	12.0 ± 2.0
1/2 Hour After Hypoxia	17.2 ± 2.8	19.7 ± 3.8	17.0 ± 3.4	17.2 ± 2.1
1 Hour	19.2 ± 3.1	18.8 ± 3.6	17.7 ± 3.7	17.9 ± 2.9
2 Hours	20.8 ± 3.8	20.6 ± 3.9	20.5 ± 4.0	21.6 ± 3.1
4 Hours	21.4 ± 3.0	21.7 ± 3.4	21.0 ± 4.4	20.2 ± 3.4
6 Hours	18.9 ± 2.1	21.1 ± 2.5	20.2 ± 3.4	19.6 ± 2.7

n is equal to 5.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

TABLE 44

PLDH ISOENZYME RATIO OF SAMPLES TAKEN SIMULTANEOUSLY FROM FOUR SAMPLING SITES IN ANIMALS RESPIRED WITH FIVE PERCENT OXYGEN FOR 15 MINUTES

Time	Carotid Artery	Right Atrium	Femoral Vein	Inferior Vena Cava
Before Hypoxia	3.05 ± 0.14*	3.12 ± 0.14	3.01 ± 0.10	2.96 ± 0.13
End of Hypoxia	2.86 ± 0.16	2.94 ± 0.17	3.06 ± 0.21	3.00 ± 0.17
1/2 Hour After Hypoxia	3.13 ± 0.19	3.04 ± 0.21	3.16 ± 0.14	2.91 ± 0.22
1 Hour	3.03 ± 0.19	3.05 ± 0.24	3.06 ± 0.13	3.04 ± 0.22
2 Hours	3.03 ± 0.26	2.87 ± 0.19	2.98 ± 0.14	3.08 ± 0.19
4 Hours	3.04 ± 0.27	3.02 ± 0.20	3.14 ± 0.18	3.17 ± 0.21
6 Hours	3.05 ± 0.17	3.20 ± 0.17	3.36 ± 0.13	3.35 ± 0.17

n is equal to 5.
* Standard Error of the Mean.

TABLE 45

PGOT AND PCPK ACTIVITY OF SAMPLES TAKEN SIMULTANEOUSLY FROM THE CAROTID ARTERY AND RIGHT ATRIUM IN CONTROL ANIMALS

Time	PGOT		PCPK	
	Carotid Artery	Right Atrium	Carotid Artery	Right Atrium
Before Hypoxia	10.7 ± 0.5*	11.1 ± 0.6	34.8 ± 3.8	35.8 ± 5.4
End of Hypoxia	10.6 ± 0.3	10.4 ± 0.3	32.3 ± 4.1	32.8 ± 3.8
1/2 Hour After Hypoxia	10.3 ± 0.3	10.8 ± 0.7	34.8 ± 4.9	34.5 ± 5.6
1 Hour	11.0 ± 0.5	11.1 ± 0.7	34.5 ± 5.6	32.7 ± 4.4
2 Hours	10.6 ± 0.7	10.6 ± 0.7	34.8 ± 5.0	32.8 ± 5.1
4 Hours	9.8 ± 0.6	10.5 ± 0.6	32.7 ± 3.0	34.0 ± 5.0
6 Hours	9.2 ± 0.7	9.1 ± 0.6	33.3 ± 3.7	34.2 ± 5.0

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

TABLE 46

PLDH ACTIVITY AND PLDH ISOENZYME RATIO OF SAMPLES TAKEN SIMULTANEOUSLY FROM THE CAROTID ARTERY AND RIGHT ATRIUM IN CONTROL ANIMALS

Time	PLDH		PLDH Isoenzyme Ratio	
	Carotid Artery	Right Atrium	Carotid Artery	Right Atrium
Before Hypoxia	11.4 ± 3.0*	11.6 ± 2.8	3.19 ± 0.11	3.20 ± 0.05
End of Hypoxia	11.7 ± 2.9	11.3 ± 3.0	3.12 ± 0.08	3.18 ± 0.10
1/2 Hour After Hypoxia	11.4 ± 2.6	11.3 ± 2.9	3.11 ± 0.11	3.22 ± 0.05
1 Hour	12.1 ± 2.7	11.7 ± 2.8	3.22 ± 0.09	3.29 ± 0.03
2 Hours	11.4 ± 2.7	11.9 ± 3.2	3.15 ± 0.05	3.35 ± 0.10
4 Hours	11.8 ± 3.2	12.1 ± 2.8	3.16 ± 0.09	3.32 ± 0.09
6 Hours	11.8 ± 2.9	11.2 ± 2.6	2.99 ± 0.07	3.32 ± 0.10

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

TABLE 47

PGOT AND PCPK ACTIVITY OF SAMPLES TAKEN SIMULTANEOUSLY FROM THE CAROTID ARTERY AND RIGHT ATRIUM IN ANIMALS RESPIRED WITH 10 PERCENT OXYGEN FOR 30 MINUTES

Time	PGOT		PCPK	
	Carotid Artery	Right Atrium	Carotid Artery	Right Atrium
Before Hypoxia	11.3 ± 0.9*	11.1 ± 1.1	14.2 ± 2.0	15.0 ± 2.2
End of Hypoxia	11.9 ± 1.2	12.2 ± 1.2	17.2 ± 1.7	16.6 ± 1.9
1/2 Hour After Hypoxia	12.2 ± 1.1	12.1 ± 1.1	18.7 ± 2.1	19.0 ± 2.8
1 Hour	12.0 ± 0.8	11.7 ± 1.0	21.2 ± 4.9	19.8 ± 3.2
2 Hours	11.5 ± 0.9	10.9 ± 0.9	25.0 ± 5.7	23.8 ± 5.0
4 Hours	11.1 ± 0.6	11.1 ± 0.7	28.7 ± 5.9	28.0 ± 6.9
6 Hours	10.4 ± 0.5	11.2 ± 0.5	30.8 ± 5.5	32.5 ± 6.6

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

TABLE 48

PLDH ACTIVITY AND PLDH ISOENZYME RATIO OF SAMPLES TAKEN SIMULTANEOUSLY FROM THE CAROTID ARTERY AND RIGHT ATRIUM IN ANIMALS RESPIRED WITH 10 PERCENT OXYGEN FOR 30 MINUTES

Time	PLDH		PLDH Isoenzyme Ratio	
	Carotid Artery	Right Atrium	Carotid Artery	Right Atrium
Before Hypoxia	9.3 ± 0.7*	10.4 ± 0.7	3.03 ± 0.12	3.04 ± 0.13
End of Hypoxia	12.2 ± 1.2	12.0 ± 1.1	3.13 ± 0.12	3.12 ± 0.09
1/2 Hour After Hypoxia	10.6 ± 0.7	11.0 ± 0.7	3.20 ± 0.15	3.01 ± 0.15
1 Hour	13.1 ± 1.0	11.5 ± 0.7	3.30 ± 0.13	3.10 ± 0.16
2 Hours	14.7 ± 1.2	12.6 ± 0.8	3.09 ± 0.16	3.22 ± 0.11
4 Hours	12.5 ± 1.4	13.2 ± 1.4	3.35 ± 0.13	3.23 ± 0.15
6 Hours	11.6 ± 1.4	13.4 ± 1.7	3.16 ± 0.19	3.35 ± 0.21

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

APPENDIX D

Means of Raw Enzyme Data: The Effect of Prednisolone or Nitroglycerin on the Plasma Enzyme Response to Hypoxia

TABLE 49

THE EFFECT OF PREDNISOLONE OR NITROGLYCERIN ON THE PGOT AND PCPK RESPONSE TO HYPOXIA

Time	PGOT			PCPK		
	21% Oxygen Prednisolone & Nitro-glycerin (Drug Control)	7.5% Oxygen Prednisolone	7.5% Oxygen Nitroglycerin	21% Oxygen Prednisolone & Nitro-glycerin (Drug Control)	7.5% Oxygen Prednisolone	7.5% Oxygen Nitroglycerin
Before Hypoxia	11.7 ± 1.4*	11.5 ± 2.4	9.8 ± 1.0	15.2 ± 1.7	19.0 ± 6.3	22.7 ± 5.1
End of Hypoxia	12.8 ± 1.7	13.7 ± 2.9	10.2 ± 0.9	14.7 ± 1.7	22.5 ± 6.4	24.7 ± 5.4
1/2 Hour After Hypoxia	13.2 ± 1.8	15.2 ± 2.8	11.7 ± 1.2	16.5 ± 2.6	30.8 ± 5.8	29.0 ± 6.0
1 Hour	13.2 ± 1.7	16.1 ± 2.9	10.5 ± 1.0	15.7 ± 2.0	33.2 ± 6.7	30.0 ± 6.3
2 Hours	13.8 ± 2.8	13.0 ± 2.9	10.4 ± 1.2	14.3 ± 1.7	34.0 ± 7.0	34.3 ± 6.4
4 Hours	13.8 ± 3.0	13.9 ± 2.8	10.2 ± 0.8	15.3 ± 2.1	34.0 ± 7.4	34.7 ± 7.3
6 Hours	13.0 ± 2.9	12.2 ± 2.3	9.8 ± 1.0	17.0 ± 2.7	33.2 ± 8.5	42.0 ± 8.6

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

TABLE 50

THE EFFECT OF PREDNISOLONE OR NITROGLYCERIN ON THE PLDH AND PLDH ISOENZYME RATIO RESPONSE TO HYPOXIA

Time	PLDH			PLDH Isoenzyme Ratio		
	21% Oxygen Prednisolone & Nitro-glycerin (Drug Control)	7.5% Oxygen Prednisolone	7.5% Oxygen Nitroglycerin	21% Oxygen Prednisolone & Nitro-glycerin (Drug Control)	7.5% Oxygen Prednisolone	7.5% Oxygen Nitroglycerin
Before Hypoxia	6.0 ± 3.8*	4.8 ± 1.3	5.3 ± 0.6	2.60 ± 0.03	2.98 ± 0.11	2.76 ± 0.08
End of Hypoxia	5.4 ± 1.4	6.6 ± 1.8	6.9 ± 1.1	2.56 ± 0.08	2.78 ± 0.10	2.81 ± 0.09
1/2 Hour After Hypoxia	6.0 ± 1.3	8.8 ± 1.7	8.4 ± 1.5	2.53 ± 0.08	3.17 ± 0.15	2.89 ± 0.14
1 Hour	5.8 ± 1.5	8.4 ± 1.1	9.0 ± 2.0	2.51 ± 0.04	3.00 ± 0.13	2.88 ± 0.13
2 Hours	6.1 ± 1.3	9.7 ± 1.7	7.6 ± 1.1	2.57 ± 0.11	3.00 ± 0.12	2.93 ± 0.15
4 Hours	8.0 ± 3.8	11.9 ± 1.7	7.9 ± 0.9	2.78 ± 0.14	3.05 ± 0.07	2.94 ± 0.02
6 Hours	7.3 ± 1.9	9.5 ± 1.6	7.1 ± 1.7	2.80 ± 0.10	3.01 ± 0.10	2.96 ± 0.08

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

APPENDIX E

Means of Raw Enzyme Data: The Effect of Succinylcholine on the Plasma Enzyme Response to Hypoxia

TABLE 51

THE EFFECT OF SUCCINYLCHOLINE ON THE PGOT AND PCPK RESPONSE TO HYPOXIA

Time	PGOT		PCPK	
	10% Oxygen Succinylcholine	10% Oxygen No Succinylcholine	10% Oxygen Succinylcholine	10% Oxygen No Succinylcholine
Before Hypoxia	11.3 ± 0.9*	7.8 ± 1.1	14.2 ± 2.0	13.7 ± 1.1
End of Hypoxia	11.9 ± 1.2	8.6 ± 0.7	17.2 ± 1.7	17.0 ± 0.9
1/2 Hour After Hypoxia	12.2 ± 1.1	8.4 ± 1.3	18.7 ± 2.1	18.2 ± 1.2
1 Hour	12.0 ± 0.8	7.6 ± 1.3	21.2 ± 4.9	18.7 ± 0.4
2 Hours	11.5 ± 0.9	8.3 ± 1.0	25.0 ± 5.7	21.5 ± 3.2
4 Hours	11.1 ± 0.6	7.8 ± 1.5	28.7 ± 5.9	25.0 ± 2.0
6 Hours	10.4 ± 0.5	8.0 ± 0.9	30.8 ± 5.5	27.2 ± 3.4

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.

TABLE 52

THE EFFECT OF SUCCINYLCHOLINE ON THE PLDH AND PLDH ISOENZYME RATIO RESPONSE TO HYPOXIA

Time	PLDH		PLDH Isoenzyme Ratio	
	10% Oxygen Succinylcholine	10% Oxygen No Succinylcholine	10% Oxygen Succinylcholine	10% Oxygen No Succinylcholine
Before Hypoxia	9.3 ± 0.7*	6.1 ± 1.8	3.03 ± 0.12	2.77 ± 0.07
End of Hypoxia	12.2 ± 1.2	7.0 ± 2.1	3.13 ± 0.12	2.67 ± 0.06
1/2 Hour After Hypoxia	10.6 ± 0.7	7.1 ± 2.1	3.20 ± 0.15	2.72 ± 0.04
1 Hour	13.1 ± 1.0	7.5 ± 1.6	3.30 ± 0.13	2.67 ± 0.03
2 Hours	14.7 ± 1.2	7.5 ± 1.9	3.09 ± 0.16	2.65 ± 0.06
4 Hours	12.5 ± 1.4	8.6 ± 1.3	3.35 ± 0.13	2.94 ± 0.10
6 Hours	11.6 ± 1.4	8.5 ± 1.5	3.16 ± 0.19	2.95 ± 0.07

n is equal to 6.
Enzyme activity is expressed as International Units per liter of plasma.
* Standard Error of the Mean.